USGA

Turfgrass and Environmental Research Online

...Using Science to Benefit Golf

Researchers at Butler University in Indianapolis, IN investigated the effects of sub-lethal levels of 2,4-D on southern leopard frogs (adult shown above). Although researchers found that acute exposure to 2,4-D reduces the activity of tadpoles and also reduces feeding activity when predators are present, they concluded that 2,4-D does not represent a particularly strong threat to amphibian larvae inhabiting golf courses where the herbicide is applied responsibly.

Volume 5, Number 16
August 15, 2006

PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 290 projects at a cost of $25 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

Editor

Jeff Nus, Ph.D.
1032 Rogers Place
Lawrence, KS 66049
jnus@usga.org
(785) 832-2300
(785) 832-9265 (fax)

Research Director

Michael P. Kenna, Ph.D.
P.O. Box 2227
Stillwater, OK 74076
mkenna@usga.org
(405) 743-3900
(405) 743-3910 (fax)

Sub-lethal Effects of 2,4-D Exposure on Golf Course Amphibians

Travis J. Ryan, Catherine M. Scott, and Brooke A. Douthitt

SUMMARY

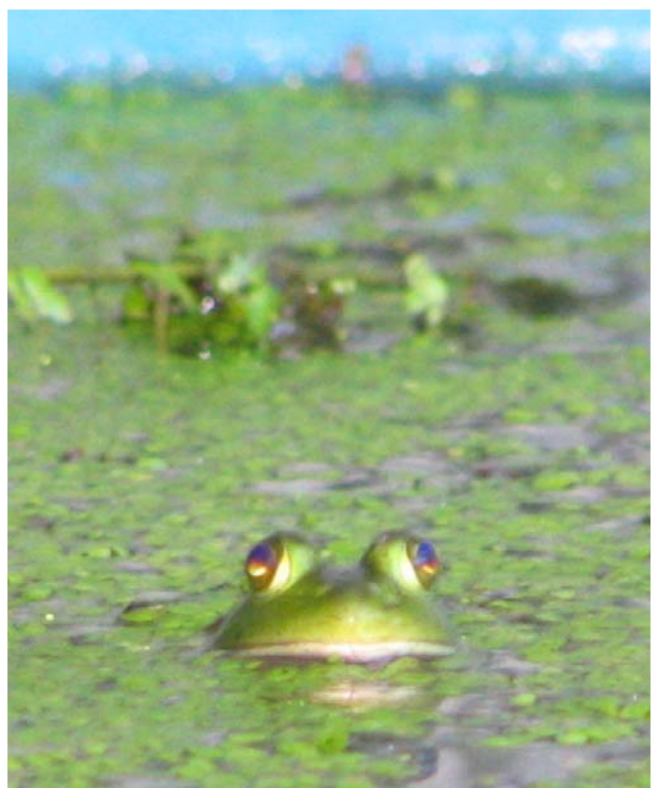
Amphibians are among the most common vertebrates inhabiting golf courses. They are frequent inhabitants of golf course wetlands, where they are likely to be episodically exposed to small quantities of herbicides in proper golf course maintenance. The goal of this study was to investigate whether amphibian larvae subjected to sub-lethal concentrations of a common herbicide used in golf course maintenance would likely result in significant life history, locomotor, or behavioral effects (e.g., changes in growth, swimming speed, or feeding ability). For these experiments we selected as models tadpoles of the southern leopard frog (*Rana sphenocephala*) and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). The study's findings include:

- Some life history traits (e.g., survival to metamorphosis) may be affected by chronic exposure at high doses, but other traits (e.g., growth, timing of metamorphosis, and size at metamorphosis) are not affected. Acute exposure is unlikely to have significant impact on life history traits.
- Acute exposure does not alter locomotor ability.
- Acute exposure to 2,4-D reduces the activity of tadpoles, and it also reduces feeding activity when predators are present.
- We conclude that 2,4-D does not represent a particularly strong threat to amphibian larvae inhabiting golf course where the herbicide is applied responsibly.

As the use of synthetic chemicals to control insects, weeds, fungi, and other "pest" species increases globally, ecologists have devoted more attention to understanding the effects of unintended exposure to pesticides on wildlife communities. This is especially true considering amphibians because pond-breeding amphibians are thought to have greater susceptibility to water-borne pesticide exposure because of their obligate aquatic larval period marked by limited mobility and relatively thin skin (1).

TRAVIS J. RYAN, Ph.D., CATHERINE M. SCOTT, B.S., BROOKE A. DOUTHITT, B.S., Department of Biological Sciences, Butler University, Indianapolis, IN

The careful application of pesticides on golf courses does not appear to eliminate amphibian communities inhabiting golf course wetlands. However, episodic contamination may result in non-lethal effects for developing amphibian larvae. Such sub-lethal effects, defined as those that impair normal biological function without resulting in direct mortality, are not necessarily less serious that outright mortality. Examples of sub-lethal effects include may reduced growth and changes other in life history events (3, 18). Additionally, the root cause of such changes may be determined at "lower" levels of biological function, such as basic physiology and behavior. For example, exposure to a toxicant may impair regular swimming function, alter activity levels, or influence feeding behavior.



Amphibians, such as frogs, represent a test species for any effect that runoff from pesticides applied to golf courses may have on the environment since they have an obligate aquatic larval period marked by limited mobility and relatively thin skin.

USGA Turfgrass and Environmental Research Online 5(16):1-14.
TGIF Record Number:

It is important to understand how pesticide exposure can affect populations of animals inhabiting golf course wetlands.

Understanding sub-lethal effects of exposure to contaminants is essential because while not responsible for direct mortality, the cumulative effects within the population may have the same general result: population inviability. Sub-lethal effects may reduce the ability of any given individual to produce offspring (via a reduction in survival or reproductive output), thus undermining the sustainability of the population. Affected populations may become "sinks" within the landscape locations where amphibians live, but are incapable of sustaining a viable population without immigration from surrounding "source" populations (17, 26).

Because sink populations may appear healthy and productive, a false sense of security may develop when monitoring efforts fail to recognize subtle sub-lethal effects. Conservation biologists and ecotoxicologists have become increasingly aware of the importance of sub-lethal effects in amphibian populations as evidenced by a recent shift away from "classic" measures of toxicity (e.g., LC_{50} tests) to more sophisticated analyses that focus on physiological (12, 24) and behavioral impacts (4, 5, 20), as well as life history characteristics (3, 18).

Dozens of pesticides are available to golf course superintendents and USGA-sponsored research investigated the manner in which a variety of pesticides affected hatching, growth, and survival of four common North American amphibian species (14). In our research, we have opted to focus on the effects of a single pesticide and a single model amphibian species in order to better understand the potential range of effects. We chose the herbicide 2,4-dichloro-phenoyacetic acid (2,4-D) for a number of critical reasons:

- 2,4-D is commonly applied during golf course maintenance. For example, an estimated 21 tons of 2,4-D are applied to golf courses annually in the South Florida Water Management District alone (19) making it one of the more commonly used herbicides on golf courses in that region. The USGA Turfgrass and Environmental Research Program has funded several research projects looking at the fate of 2,4-D and other pesticides applied on golf courses (15). These studies, focusing on the mobility of pesticides, demonstrated that concentration of 2,4-D in golf course wetlands is not unlikely (27, 30). The potential for aquatic contamination represents an unexplored threat for amphibian larvae and other members of lentic aquatic communities.
- Despite being one of "the most widely researched herbicides in the world" (23), the effects of 2,4-D exposure on amphibians are poorly understood at all life-cycle stages. Some standard toxicological studies of 2,4-D and related compounds have been preformed on adult newts (31, 32), but not embryonic or larval amphibians which are likely more susceptible to exposure. Most toxicological studies of 2,4-D have featured

Predators, such as the green sunfish shown above, can produce chemical signals that will reduce the movement of potential prey, such as tadppoles of the southern leopard frog used in this study.

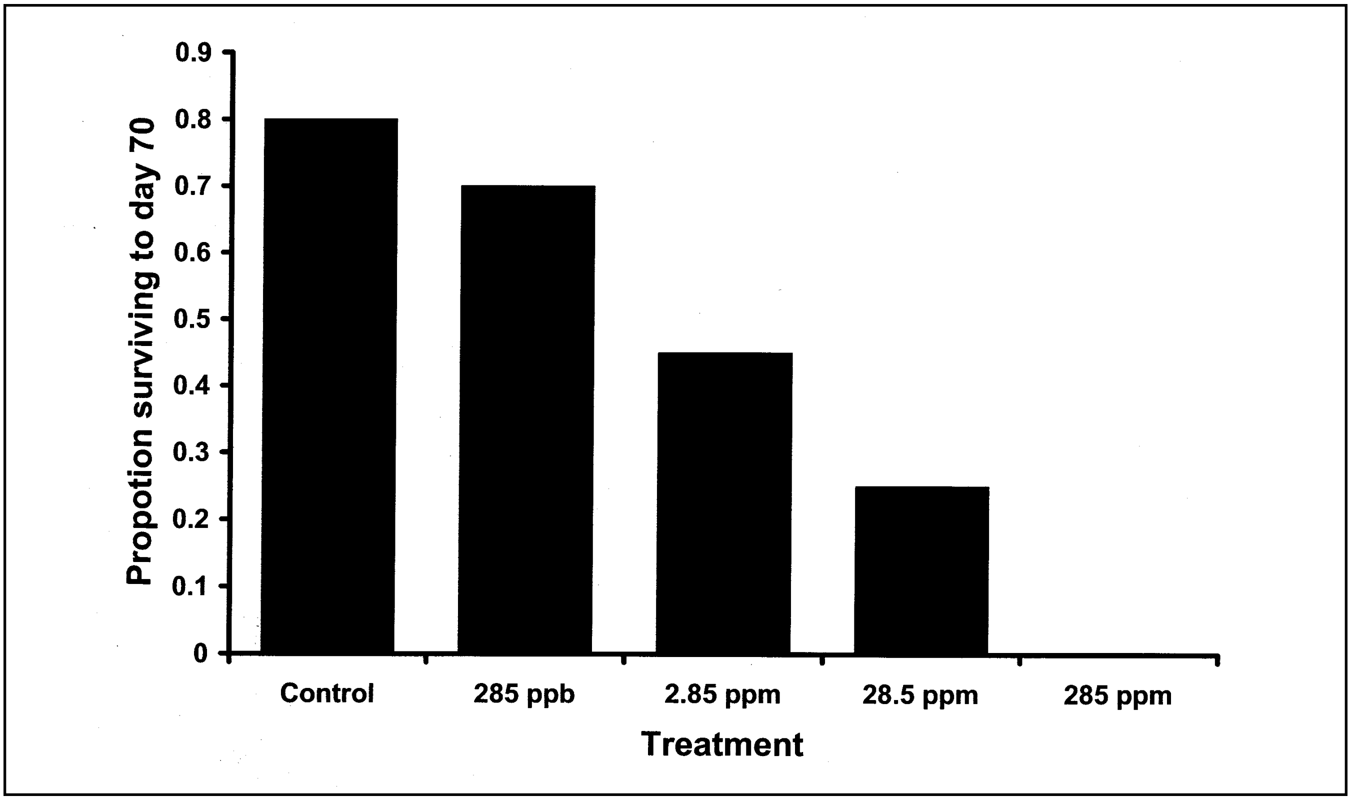


Figure 1. Effect of chronic exposure to 2,4-D on survivorship of southern leopard frog tadpoles

mammalian models and thus the reputation of the pesticide as ecologically benign (23) may be unwarranted with regard to amphibians.

● 2,4-D has wide application in the US, Canada, and the rest of the world. It is the third most commonly applied pesticide in the US and is the most widely used herbicide worldwide (23). Thus our research may have relevance not only for golf course maintenance and the golf industry, but a wider applicability regarding the effects of 2,4-D contamination on amphibian biology.

We used the southern leopard frog (*Rana sphenocephala*) as the model amphibian for this research. *Rana sphenocephala* has a relatively brief larval period (< 90 days), similar to many amphibians breeding in golf course wetlands, and relatives of *R. sphenocephala* in the leopard frog (*R. pipiens*) complex are distributed throughout the US, giving our study wide geographic applicability. Members of the genus *Rana* are commonly used in various stages of development in diverse research contexts [e.g., population biology and metamorphic timing (25), ecotoxicology (3, 4, 11), conservation biology (6)]. Finally, frogs of the genus *Rana* are more commonly used in ecotoxicology studies than any other North American genus, giving our results a broad basis for comparison.

Methods

Chronic Exposure Study

The goal of our research was to look at the sub-lethal effects of 2,4-D on *R. sphenocephala* in an ecologically realistic manner with regards to golf course wetlands. Because the herbicide has a fairly short half-life (23, 28) and is most likely to appear in golf course wetlands at high concentrations only under particular conditions (e.g., application near wetlands prior to heavy rainfall), this warrants a focus on acute rather than chronic exposure. However, we conducted a chronic exposure study to determine the range of effects on life history characteristics (e.g., growth, metamorphosis, and survival) under a worse-case scenario. We also used thus study to select concentrations to be used in the locomotor and behavioral studies.

We obtained egg masses of *R. sphenocephala* from Carolina Bays in Aiken County, South Carolina. After hatching, we randomly selected 100 tadpoles and housed them in 2-liter beakers, each filled with approximately 1600 mL of well-aged water. We used a control (no 2,4-D) and four concentrations of 2,4-D that range from 285 ppb (= 0.285 ppm; lowest) to (285 ppm; highest) with an order of magnitude (factor of 10) between each concentration. We recoded the mass of the tadpoles and 20 were assigned to each treatment with a static exposure (i.e., at each weekly water change the assigned 2,4-D concentration was renewed). The mass of the tadpoles was recorded 21 days after the initiation of the experiment, and roughly every 14 days thereafter. The tadpoles were fed a diet of ground fish food.

The tadpoles were checked daily throughout the experiment for mortality and signs indicating the beginning and completion of metamorphosis (forelimb eruption and tail resorption, respectively). We stopped recording the mass of tadpoles after day 68, as after this point tadpoles began metamorphosis and growth generally ceases during this process. The experiment was continued until all survivors completed metamorphosis.

<u>*Locomotor Study*</u>

To determine how acute exposure to 2,4-D influences locomotor performance, we measured the swimming speed of tadpoles following a 24-hour exposure to 2,4-D. Three egg masses of *R. sphenocephala* were collected from a natural breeding site in Aiken County, South Carolina. After hatching, 72 tadpoles were randomly selected and assigned to individual glass containers containing approximately 1,550 mL aged tap water. Tadpoles were fed *ad libitum* every three days and water was changed weekly.

The tadpoles were exposed to 2,4-D 24 hours prior to the swimming trials. A total of 18 individuals were selected randomly and exposed to the experimental treatments: either one of three

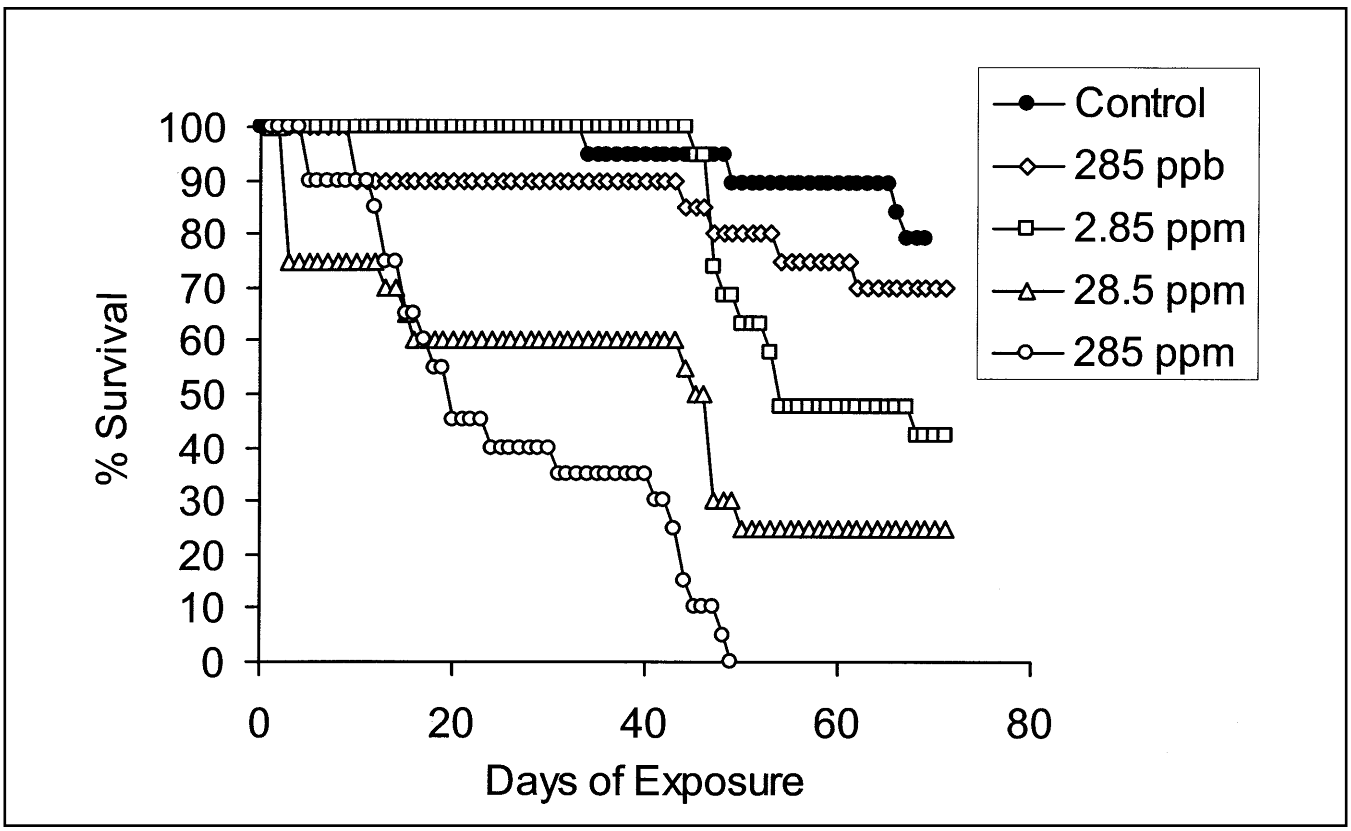


Figure 2. Survivorship of southern leopard frog tadpoles exposed to 2,4-D over more than 70 days

different concentrations of 2,4-D [150 ppm (high), 15 ppm (medium), 1.5 ppm (low), or control (no 2,4-D). Approximately 24 hours after exposure, the swimming performance of tadpoles was assessed using a linear swimming arena (100 cm long x 12 cm wide x 6.5 cm deep) marked in 1 cm intervals. The arena was filled with aged tap water and narrowed to 6 cm wide during the trials to limit lateral movement and discourage backwards motion.

Tadpoles were introduced to the arena in a holding area where they were permitted to acclimate for approximately two minutes. At the start of each trial, a barrier separating the holding area and the swimming track was removed. If a tadpole failed to initiate swimming spontaneously, it was gently prodded near the base of the tail with a blunt probe. If the tadpole ceased movement for two seconds prior to completing the length of the track, it was again gently prodded. Each trial ended when the tadpole had completed swimming the distance of 100 cm. At the end of the trial, the tadpole was placed in a holding container filled with 200 mL aged tap water and allowed to rest for at least one hour prior to the start of the next trail. Each tadpole swam a total of 3 trials. Swimming trials were conducted approximately 45 days after hatching.

Each swimming trial was recorded using a digital video camera. This permitted a frame-by-frame analysis of swimming performance, allowing us to accurately record start and stop times and positions on the swimming track. We recorded the number of bursts (discrete swimming events required to complete the 100 cm track), burst speed (the rate of movement during each burst in cm/sec), and total swimming speed (cm/sec averaged over the 100-cm track length). Appropriate statistical analyses were conducted to determine the effect of acute 2,4-D exposure on swimming speed.

<u>*Behavior Studies*</u>

We observed the behavior of tadpoles following acute exposure to 2,4-D in order to determine whether activity, feeding performance, or habitat use are impacted. We obtained *R. spheno-*

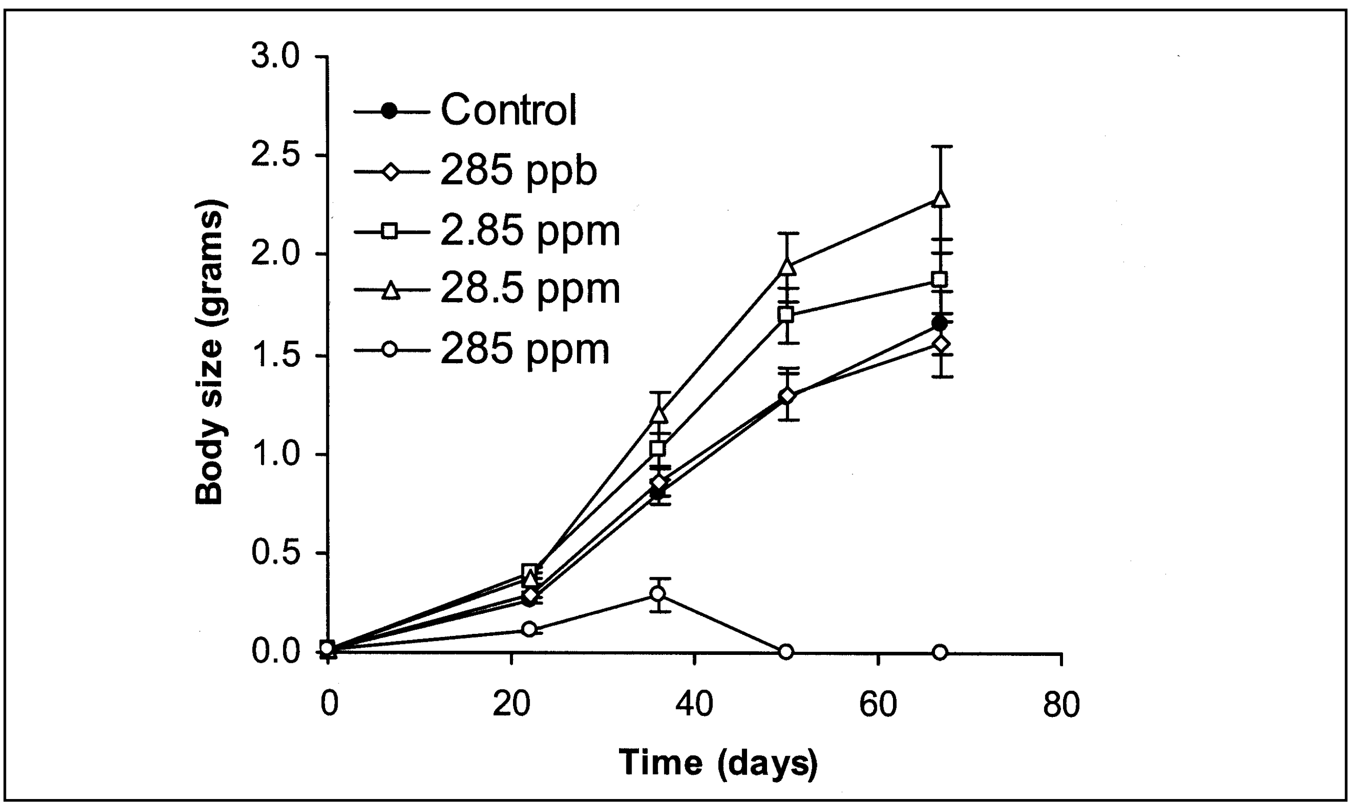


Figure 3. Growth of southern leopard frog tadpoles exposed to 2,4-D over more than 70 days

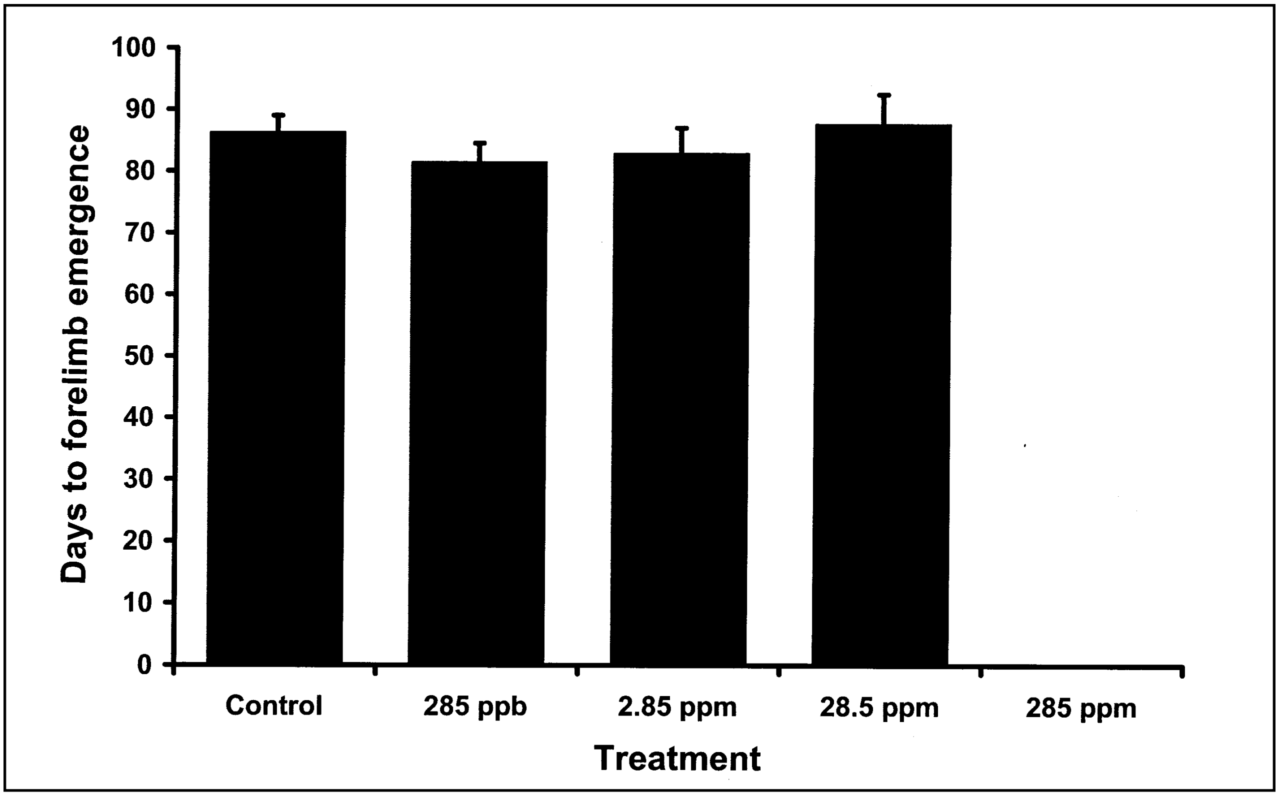


Figure 4. Effect of chronic exposure to 2,4-D on forelimb emergence of southern leopard frog tadpoles. Bars a treatment means and error bars are equal to one standard error.

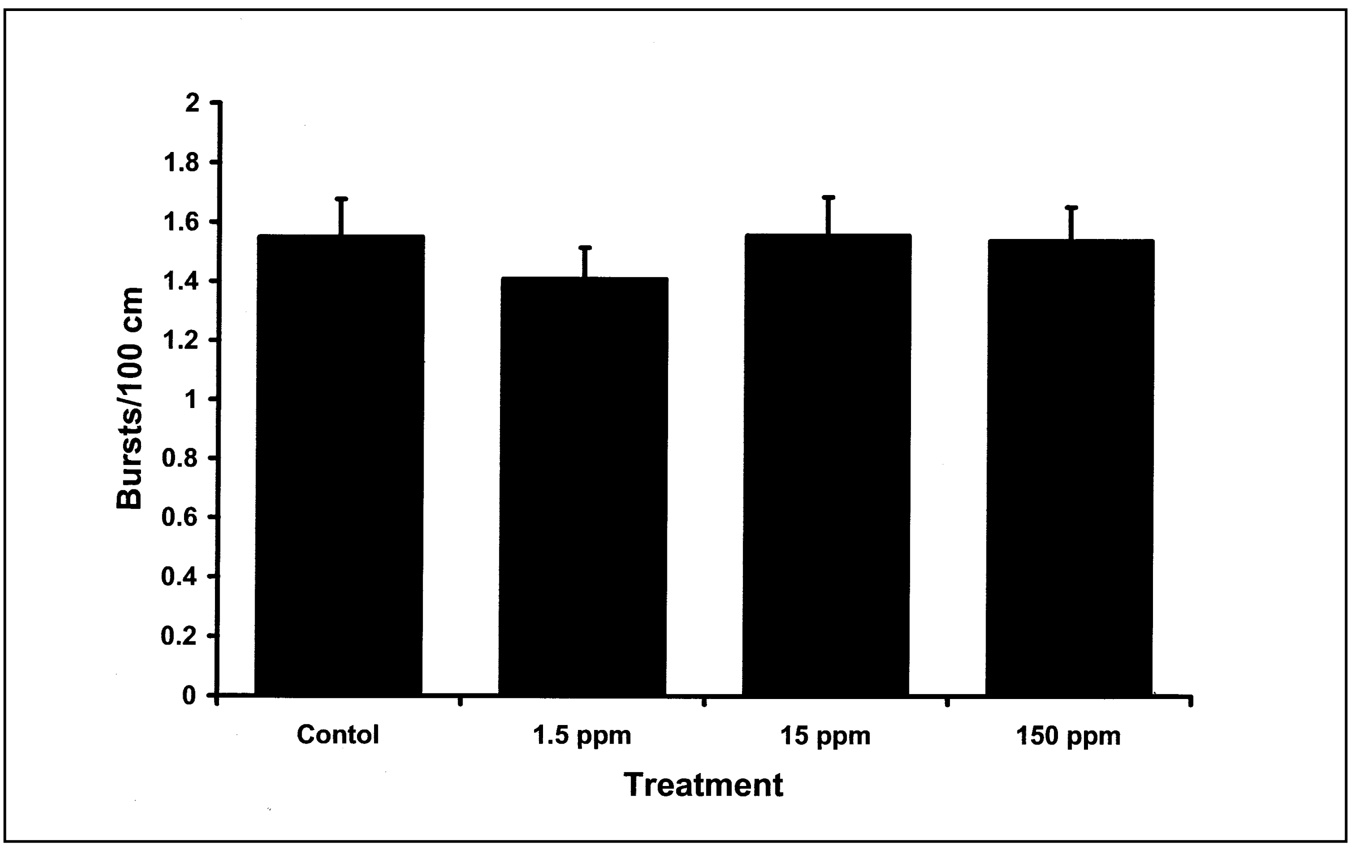


Figure 5. Effect of acute (24 hour) exposure to 2,4-D on the number of swimming bursts required for southern leopard frog tadpoles to cover the 100 cm swimming track. Bars a treatment means and error bars are equal to one standard error.

cephala as above. Eggs were hatched and tadpoles were kept in multiple 65-liter polyethylene containers and fed ground fish food. Tadpoles at developmental stage 33-3523 and of similar size were placed in individual glass containers containing 1,550 mL aged tap water. The water was changed every third day and feeding remained *ad libitum*. Prior to the behavioral experiments, the water was changed and tadpoles were deprived of food for 72 hours. Food deprivation was done to insure that tadpoles would be hungry and likely to feed during the trials (13). Exposure levels were high (150 ppm) and low (1.5 ppm) as in the swimming performance experiment.

Following the 24-hour exposure period, all behavioral observations were made in 38-liter aquaria. We used four aquaria simultaneously during observation periods. The aquaria were placed on a grid marked with 1.27-cm squares so an unbiased location of the tadpoles could be determined. Each aquarium also contained a refugium located in the center of the tank. The refugium was made from PVC pipe measuring approximately 8 cm. Two pieces of fiberglass window screening, approximately 10 cm by 10 cm, were attached in a bunch with many folds at either ends of the refuge with a rubber band. We placed a digital video recorder above the aquaria to record tadpole behavior during the 25-minute observation period permitting us to record the trials more expeditiously and allow for a careful analysis of behavior.

At the start of each observation period, we placed an individual tadpole into the center of the aquaria. After a two-minute acclimation time, we turned on the video camera and recorded behavior for 25 minutes. During the observation period, we vacated the room and closed it off to avoid the bias of observer presence. The trials were conducted over five days (presence of food experiment) and 6 days (presence of predator experiment) with three trials of four individuals each recorded on a single day. Between each trial, we rinsed the aquaria thoroughly to eliminate chemical cues from previous groups.

We conducted two experiments, one

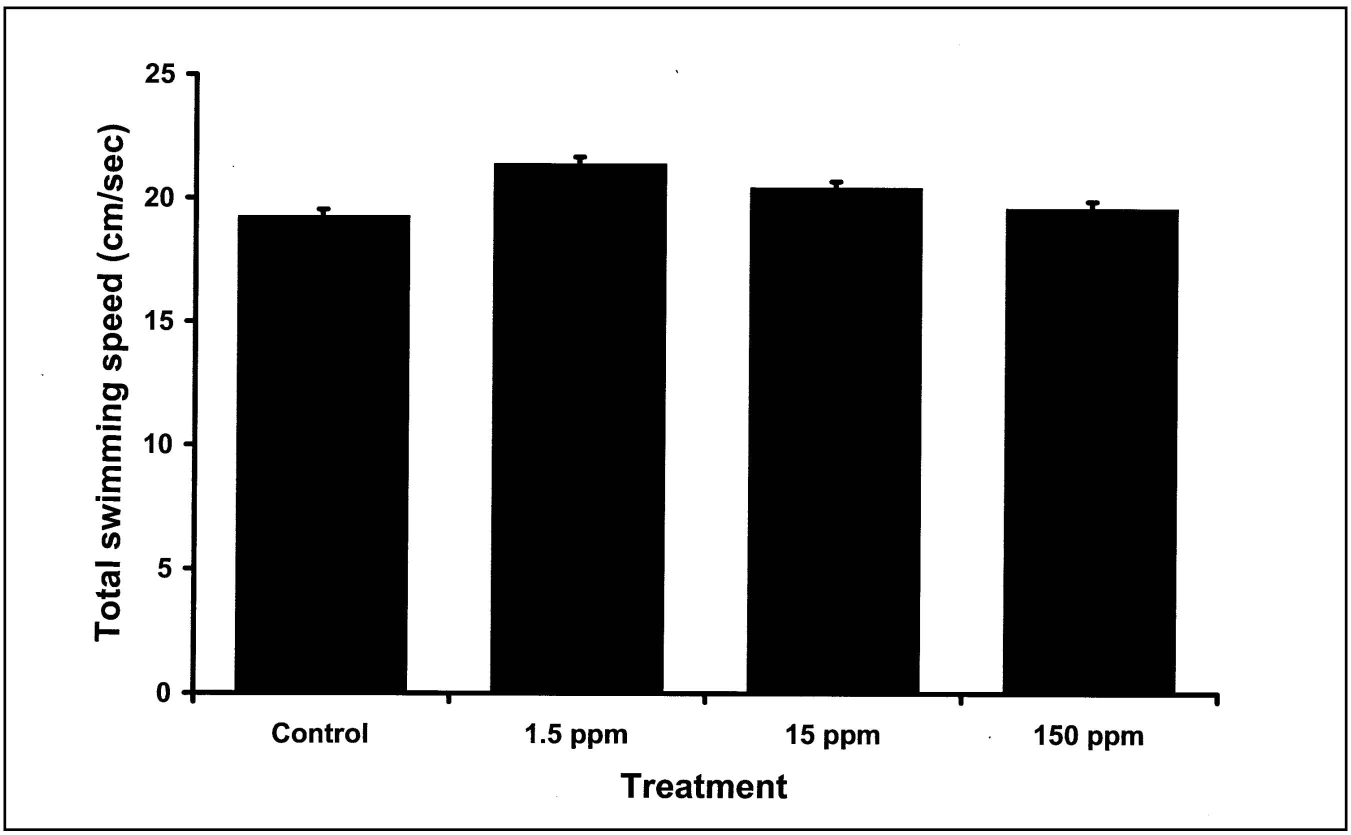


Figure 6. Effect of acute (24 hour) exposure to 2,4-D on the swimming speed of southern leopard frog tadpoles over a distance of 100 cm. Bars a treatment means and error bars are equal to one standard error.

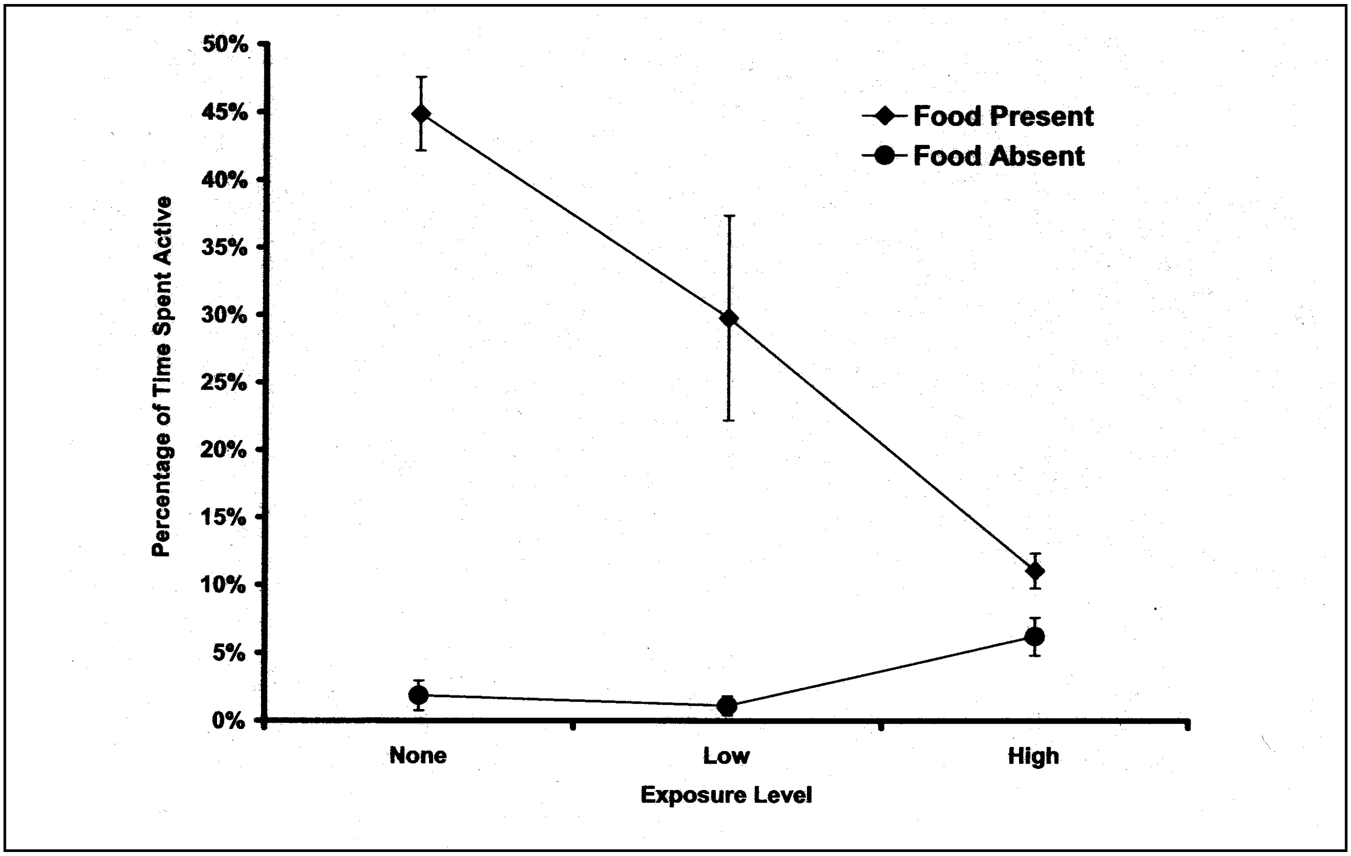


Figure 7. Time southern leopard frogtadpoles spent active with a source of food present and absent at different 2, 4-D exposure levels. Points are treatment means with error bars equal to one standard error.

observing the effects of food on behavior and the second observing the effect of the presence of a predator on behavior. We placed half of the tadpoles exposed to each 2,4-D level (control, low, high) in aquaria containing approximately 5.5 L of aged tap water with ground fish food. The remaining tadpoles were placed in aquaria containing 5.5 L 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 conducted a second test. Half of the tadpoles exposed to each 2,4-D level (control, low, high) were placed in aquaria containing approximately 5.5 L aged tap water in which predator chemical cues were present. Tadpoles rely on chemical cues released from the fish as predator presence (29) and chemical cues released from other tadpoles as a warning signal (7, 16). "Fish water" was used in the predator treatment as the chemical cues. Four green sunfish (*Lepomis cyanellus*) were fed a diet of *R. sphenocephala* tadpoles not used in the experiment. The fish spent a minimum of 72 hours in a separate polyethylene container containing approximately 65 L of aerated aged tap water. The remaining half of the tadpoles exposed to each 2,4-D level were placed in aquaria containing approximately 5.5 L of 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. Activity was defined as any movement of the tadpole even if a change in location was not observed. Feeding was defined as a movement to the surface of the water in which the disappearance of food flakes was observed. Location was recorded by observing tadpole location relative to the grid below the aquariums.

Tadpoles within two squares of the edge of the aquaria were recorded as being at the edge. Within one square of the refugium and in the refugium was recorded as refuge use, and any other position was regarded as open. Analysis of variance (ANOVA) models were used to determine the effects of exposure to 2,4-D on feeding behavior in the presence and absence of food and in the presence and absence of chemical cues. Tukey simultaneous pairwise comparisons were used to identify significant differences between exposure levels and among combinations of exposure levels and presence of food or chemical cues when significant effects were observed.

Results

Chronic Exposure Study

Long-term exposure to 2,4-D had an effect on survival to the end of the experiment (Figure 1). However, it is important to note that mortality, even at the highest concentration was not immediate. Nearly 20 days passed before the survivorship in the 285 ppm treatment was 50% (Figure 2). In the 2.85 ppm treatment, survivorship did not dip below 50% of the control group by the end of the study. While growth was compromised in the highest concentration group, an analysis of the control treatment and three lower concentrations showed that growth (body size in grams at day 68) was not impacted by chronic exposure to 2,4-D (Figure 3). Furthermore, there was no effect on the timing of metamorphosis (Figure 4), excluding 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 (Figure 5) and total sprint speed in cm/sec (Figure 6).

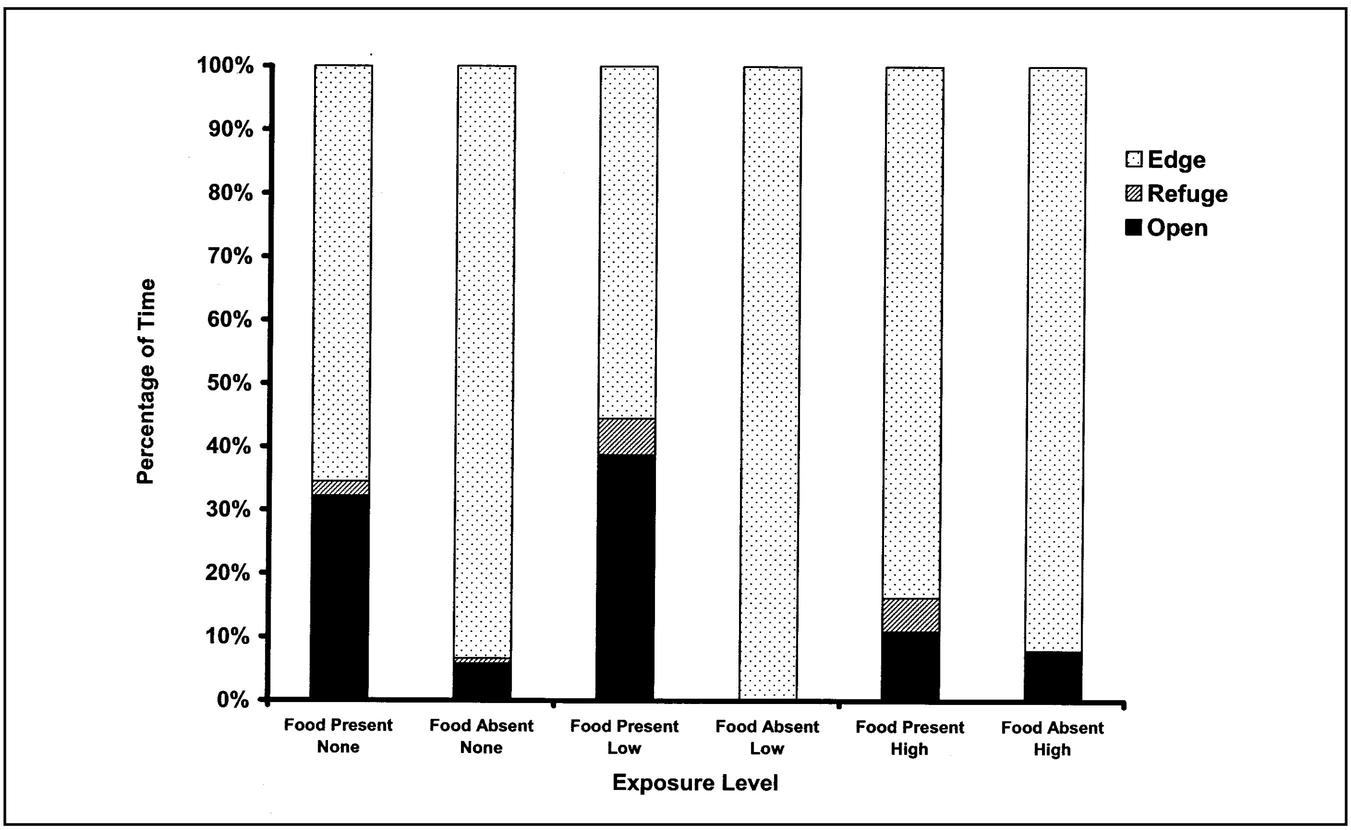


Figure 8. Time southern leopard frog tadpoles spent in different locations of the tank (open, refuge, and edge) with a source of food present and absent at different 2,4-D exposure levels.

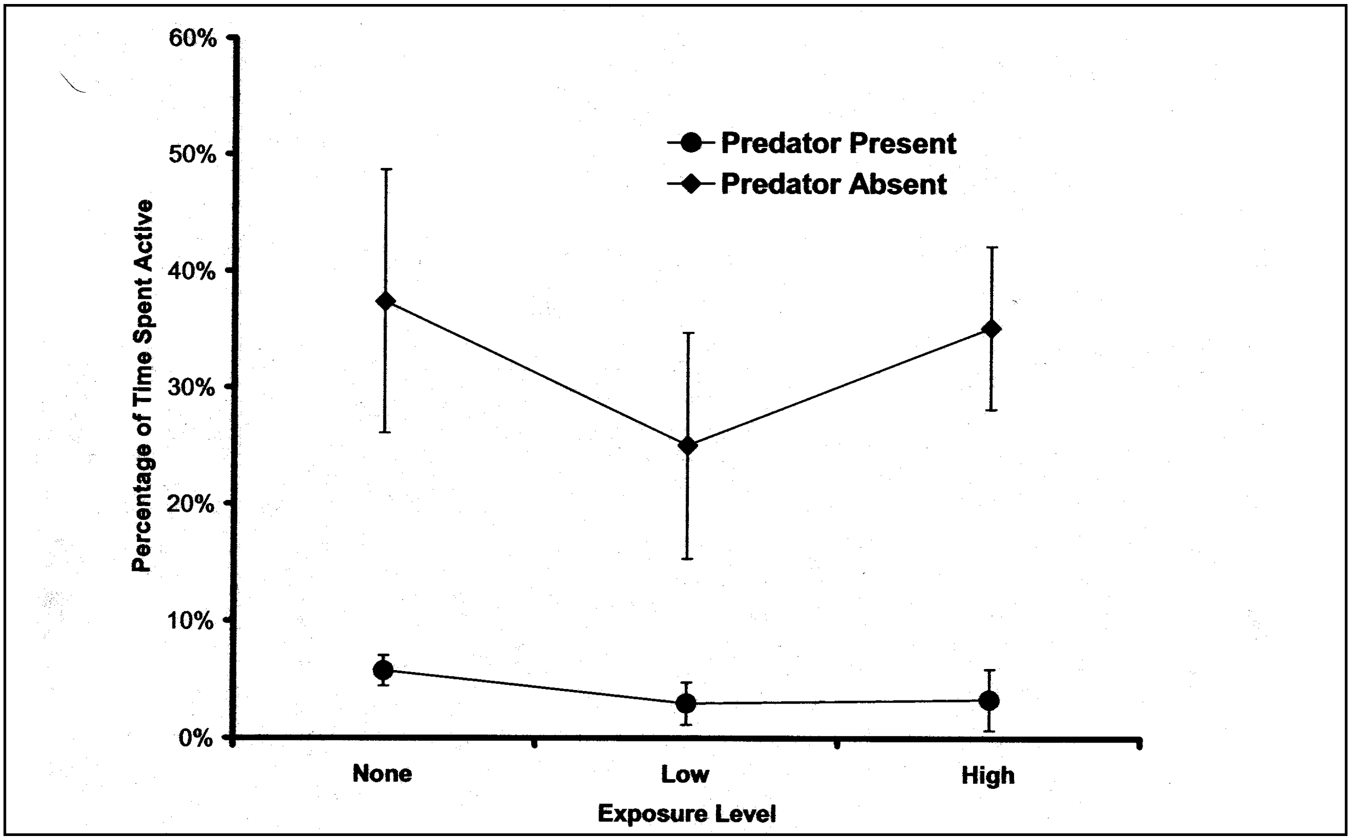


Figure 9. Time southern leopard frog tadpoles spent feeding with predator cues present and absent at different 2,4-D exposure levels. Points are treatment means with error bars equal to one standard error.

Behavior Study: Effect of Food

2,4-D exposure levels had no significant effect on activity, feeding, or habitat use. 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 (Figure 7). The interaction is complex, but may be summarized as follows:

- When food was present unexposed tadpoles were more active than those exposed to low concentrations of 2,4-D, which were more active than those exposed to high concentrations of 2,4-D.
- Under control (unexposed) conditions, tadpole activity decreased significantly when food was not present.
- Activity did not change regardless of whether food was present for tadpoles exposed to 2,4-D at a concentration of 150 ppm, as they already had reduced levels of activity, even when food was present.
- After exposure to a low concentration (1.5 ppm) of 2,4-D, tadpoles had a response intermediate between the control and high concentration treatments.
- The presence of food in the experimental tanks had a significant effect on the "habitat" use within the aquaria regardless of 2,4-D exposure level (Figure 8). When food was present, time spent in the open increased significantly.
- Time spent in the refuge was also significantly higher when food was present, thus decreasing time spent at the edges of the aquaria.

Behavior Study: Effect of Predator Cues

Exposure to 2,4-D had no significant effect on activity when tadpole were in the presence of predator cues. However, exposure to 2,4-D did affect tadpole feeding behavior (Figure 9). Tadpoles exposed to low levels of 2,4-D fed considerably less than those not exposed to 2,4-D but not significantly different from tadpoles experi-

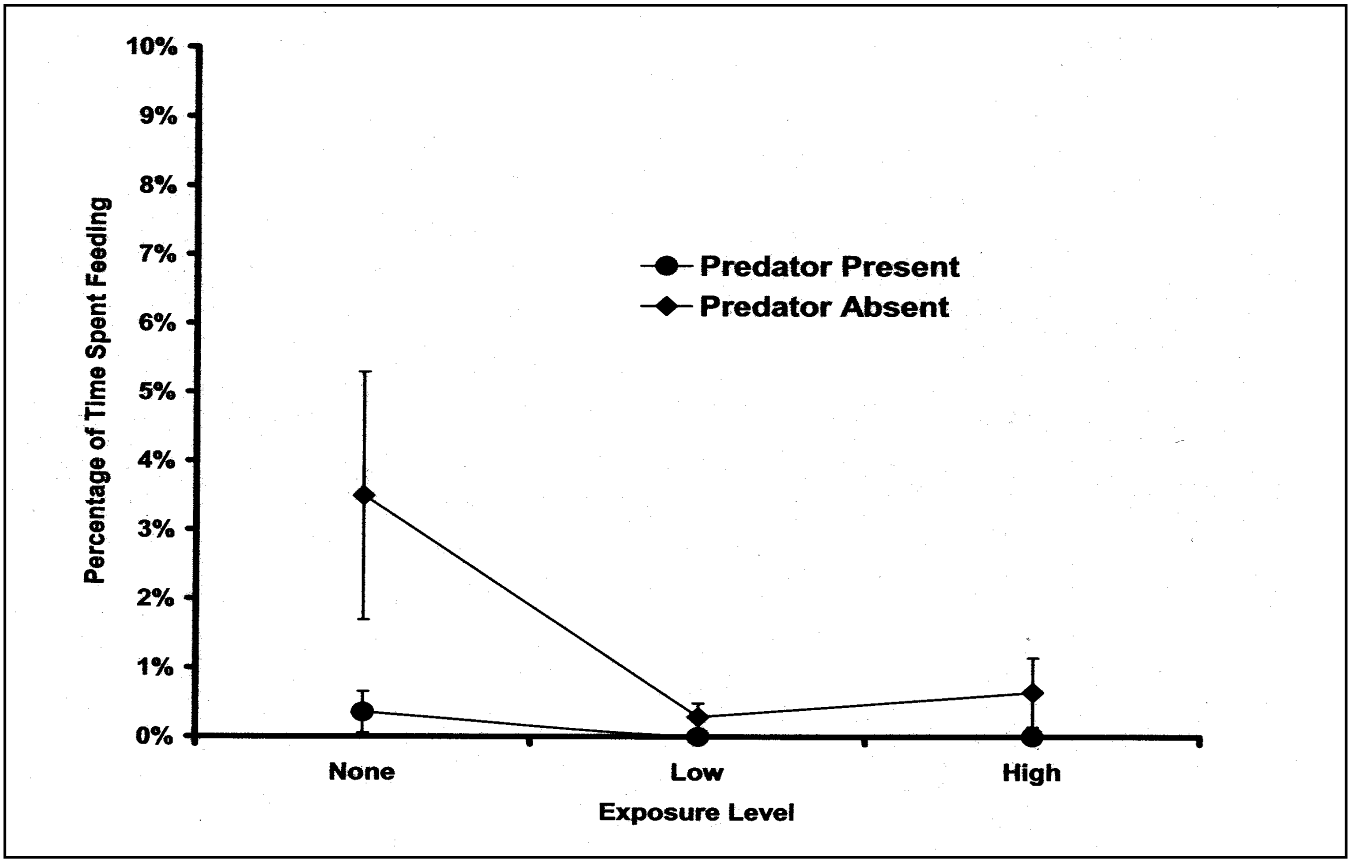


Figure 10. Time southern leopard frog tadpoles spent active with predator cues present and absent at different 2,4-D exposure levels. Points are treatment means with error bars equal to one standard error.

encing high exposure. Only a marginally significant difference in time spent feeding was observed between high 2,4-D exposure and absence of 2,4-D. As in the first test, exposure to 2,4-D had no significant effect on tadpole location.

The presence of a predator's chemical cues significantly reduced activity (Figure 10) and feeding (Figure 9) compared to when no chemical cues were present, but had no significant effect on tadpole location.

Discussion

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 *R. spenocephala.* This is in contrast to many other pesticides that have been shown to slow growth, increase mortality, and impair metamorphosis in a wide array of amphibians after a short period of exposure (1). For example, USGA Green Section-funded research by Jim Howard (14) and his colleagues showed that *R. spenocephala* survival was affected by exposure to a concentration one-half the LC_{50} (concentration found to be lethal to 50% of exposed individuals) for three common insecticides, and exposure to 1/10th the LC_{50} was sufficient to reduce growth significantly.

We did not use LC_{50} values in this research as the concentrations necessary to obtain clinical values would have been so high as to be ecologically inconsequent (e.g., at 285 ppm the time for 50% of individuals to die was 240 hours rather than 48 hours as is expected in clinical laboratory trials). In addition to the relative low toxicity of 2,4-D to *R. spehenocephala,* is the fact that this herbicide breaks down rather quickly in aquatic environments (23, 28). These characteristics taken together suggest that 2,4-D represents a relatively low level threat in terms of direct mortality to amphibian larvae.

This is consistent with a recent large-scale experiment that found when 2,4-D is applied directly to aquatic systems at a concentration of 0.117 ml/m^2 (equivalent to a recommended application), there was no significant effect on amphibian mortality or aquatic community structure (21). It is worth noting that a similar application of other pesticides did not elicit such benign responses from tadpoles and other animals comprising the experimental aquatic communities.

The absence of direct mortality and the resultant changes in community organization, however, are only part of the story with regards to the potential detrimental effects of pesticide exposure in amphibian larvae. The sub-lethal 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 sub-lethal doses of other common pesticides, such as carbaryl (5) and cypermethrin (9).

We also investigated the effect of sub-lethal 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 (i.e., they decreased activity), but the exposed tadpoles also had decreased levels of feeding in the absence of predators.

Because of the lethality and mutagenic properties of other environmental contaminants, the action of some pesticides on non-target species is understood. For example, the insecticide carbaryl acts as an acetylcholinesterase inhibitor (1), disrupting the neurotransmitter acetylcholine, thereby explaining interrupted locomortor activities. The herbicide atrazine acts as an endocrine disruptor in amphibians and results in reduced testosterone production in male frogs (10).

Due to the low toxicity of 2,4-D, the activity of the compound in amphibians is not understood. Decreased movement and feeding levels may be of concern as depression in these activities are likely to result in decreased growth and thus longer time to metamorphosis and increased exposure to predators. Indeed, recent research in the field of amphibian ecology highlights the role of interactions between environmental contaminants and community structure as being important in determining the true ecological effect of chemically intensive practices (2, 22).

For golf course superintendents, the risk of detrimental effects to larval amphibian populations through exposure to 2,4-D appears to be very low given the low toxicity of the compound and the relatively subtle sublethal effects, especially if proper attention is given to the appropriate application of the herbicide.

Acknowledgements

The authors would like to thank the United States Golf Association'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.

Literature Cited

1. Boone, M. D., and C. M. Bridges. 2003. Effects of pesticides on amphibian populations. Pages 152-167. *In* Amphibian Conservation. R. D. Semlitsch (ed.). Smithsonian Institution Press, Washington, D. C.

2. Boone, M. D., and R. D. Semlitsch. 2003. Interactions of bullfrog tadpole predators and an insecticide: predation release and facilitation. *Oecologia* 422:610-616.

3. Bridges, C. M. 2000. Predator-prey interactions between two amphibian species: effects of

insecticide exposure. *Archives of Environmental Contamination & Toxicology* 39:91-96.

4. Bridges, C. M. 1999. Effects of a pesticide on tadpole activity and predator avoidance behavior. *Journal of Herpetology* 33: 303-306.

5. Bridges, C. M. 1997. Tadpole swimming performance and activity affected by acute exposure to sublethal levels of carbaryl. *Environmental Toxicology and Chemistry* 16:1935-1939.

6. Bridges, C. M., and R. D. Semlitsch. 2000. Explaining differential declines of amphibian species: variation in pesticide sensitivity of tadpoles among and within species of Ranidae. *Conservation Biology* 14:1490-1499.

7. Chivers, D. P., J. M. Kiesecker, A. Marco, E. L. Wildy, and A. R. Blaustein. 1999. Shifts in life history as a response to predation in western toads (*Bufo boreas*). *Journal of Chemical Ecology* 25: 2455-2464.

8. Gosner, N. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183-190.

9. Greulich, K., and S. Pflugmacher. 2003. Differences in susceptibility of various life stages of amphibians to pesticide exposure. *Aquatic Toxicology* 65:329-336.

10. Hayes, T., K. Haston, M. Tusi, A. Hoang, C. Haeffele, and A. Vonk. 2003. Atrazine-induced hermaphroditism at 0.1 PPB in American frogs (*Rana pipiens)*: laboratory and field evidence. *Environmental Health Perspectives* 111:568-575.

11. Hopkins, W.A., J. K. Ray, and J.D. Congdon. 2000. Incidence and impact of axial malformations in bullfrog larvae (*Rana catesbeiana*) developing in sites polluted by a coal burning power plant. *Environmental Toxicology and Chemistry* 19:862-868.

12. Hopkins, W. A., M. T. Mendonca, and J. D. Congdon. 1999. Responsiveness of the hypothalamo-pituitary-interrenal axis in an amphibian (*Bufo terrestris*) exposed to coal combustion wastes. *Comparative Biochemistry and Physiology* Part C 122:191-196.

13. Horat, P., and R. D. Semlitsch. 1994. Effects of predation risk and hunger on the behavior of two species of tadpoles. *Behavioral Ecology and Sociobiology* 34:393-401.

14. Howard, J. H., S. E. Julian, and J. Ferrigan. 2002. Golf course design and maintenance: impacts on amphibians. *USGA Turfgrass and Environmental Research Online* 1(6):1-21 (TGIF Record 82797)

15. Kenna, M. P. 1995. What happens to pesticides applied to golf courses? *USGA Green Section Record* 33:1-9. (TGIF Record 32638)

16. Kiesecker, J.M., D. P. Chivers, M. Anderson, and A. R. Blaustein. 2002. Effects of predator diet on life history shifts of red-legged frogs, *Rana aurora*. *Journal of Chemical Ecology* 28: 1007-1016.

17. Levin, D. A. 1995. Metapopulations: an arena for local speciation. *Journal of Evolutionary Biology* 8:635-644.

18. Marian, M. P., V. Arul, and T. J. Pandian. 1983. Acute and chronic effects of carbaryl on survival, growth, and metamorphosis in the bullfrog (*Rana tigrina*). *Archives of Environmental Contamination and Toxicology* 12:271-275.

19. Miles, C. J., and R. J. Pfeuffer. 1997. Pesticides in canals of south Florida. *Archives of Environmental Contamination and Toxicology* 32:337-345. (TGIF Record 57188)

20. Raimondo, S. M., C. L. Rowe, and J. D. Congdon. 1998. Exposure to coal ash impacts swimming performance and predator avoidance in larval bullfrogs (*Rana catesbeiana*). *Journal of Herpetology* 32:289-292.

21. Relyea, R. A. 2005. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecological Applications* 15:618-627. (TGIF REcord 114046)

22. Relyea, R. A. 2004. Synergistic impacts of malathion and predatory stress on six species of North American tadpoles. *Environmental Toxicology and Chemistry* 23:1080-1084.

23. Rhoades, R. Industry task force II on 2,4-D research data [website]; http://www.24d.org [Accessed 29 January 2004]. (TGIF Record 108884)

24. Rowe, C. L., O. M. Kinney, R. D. Nagle, and J. D. Congdon. 1998. Elevated maintenance costs in an Anuran (*Rana catesbeiana*) exposed to a mixture of trace elements during the embryonic and early larval periods. *Physiological Zoology* 71:27-35.

25. Ryan, T. J., and C. T. Winne. Effects of hydroperiod on metamorphosis in *Rana sphenocephala*. *American Midland Naturalist* 145:46-53.

26. Semlitsch, R. D., and J. R. Bodie. 1998. Are small, isolated wetlands expendable? *Conservation Biology* 12:1129-1133. (TGIF Record 82409)

27. Snow, J. T. 1996. Loss of nitrogen and pesticides from turf via leaching and runoff. Presentation made at the Australian Turfgrass Conference, March 1996. (TGIF Record 37752)

28. Spectrum Laboratories Inc. Spectrum Laboratories: Chemical Fact Sheet - Cas# 94757 [Website]; http://www.speclab.com/compound/c94757.htm [Accessed 18 June 2006].

29. Stauffer, H.P., and R. D. Semlitsch. 1993. Effects of visual, chemical and tactile cues of fish on the behavioral responses of tadpoles. *Animal Behavior* 46: 355-364.

30. Watschke, T. L., S. Harrison, and G. W. Hamilton. 1989. Does fertilizer/pesticide use on a golf course put water resources in peril? *USGA Green Section Record* 27:5-8. (TGIF Record 14834)

31. Zaffaroni, N. P., T. Zavanella, M. L. Ferrari, and E. Arias. 1986. The toxicity of 2,4-dichlorophenoxyacetic acid on the adult crested newt. *Environmental Research* 41:79-87.

32. 18. Zaffaroni, N. P., T. Zavanella, M. L. Ferrari, and E. Arias. 1986. The toxicity of 2-methyl-4-chlorophenoxyacetic acid on the adult crested newt. *Environmental Research* 41:201-206.