

# The interactive effects of UV-B and insecticide exposure on tadpole survival, growth and development

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Received 6 April 2002; received in revised form 14 September 2002; accepted 20 October 2002

## Abstract

Because declines within amphibian populations can seldom be attributed to a single cause, it is important to focus on multiple stressors, both natural and anthropogenic. Variables such as UV-B radiation and chemical contamination can interact with one another in ways that might not be predicted from single-factor studies. We exposed southern leopard frog (*Rana sphenoccephala*) tadpoles to the insecticide carbaryl and varying intensities of UV-B radiation in artificial ponds and examined their effects on survival, size at metamorphosis, and the duration of the larval period. Tadpole survival to metamorphosis was positively influenced by UV-B intensity. Tadpoles in ponds exposed to carbaryl contained over three times more algae and yielded larger metamorphs than control ponds. Although previous laboratory studies have indicated carbaryl becomes more toxic in the presence of UV-B, we did not find such an effect, perhaps because of the protection afforded by dissolved organic carbon within the ponds. Our research emphasizes the importance of conducting field studies to more accurately predict what occurs under a natural setting.

Published by Elsevier Science Ltd.

**Keywords:** Insecticide; Metamorphosis; Multiple stressors; Tadpoles; UV-B radiation

## 1. Introduction

Localized declines of amphibians have been widely reported (Blaustein and Wake, 1990; Houlihan et al., 2000), yet no single factor has been determined to consistently cause declines. Many factors have been proffered as having contributed to declines including parasites and disease (Carey et al., 1999), habitat loss and destruction (Blaustein et al., 1994), introduction of exotic species (Kiesecker and Blaustein, 1997), ultraviolet radiation (Broomhall et al., 2000), and environmental contamination (Davidson et al., 2001). Single-factor explanations simply may not be sufficient to explain this widespread phenomenon. Stallard (2001), Carey et al. (2001), and Laurance (1996) sought correlations between environmental variables and amphibian declines, but failed to find a satisfactory explanation for such losses, and suggested several interacting mechanisms may be the source of population declines. Amphibians in natural communities typically accommodate a number of potentially harmful natural factors through-

out their lifecycle (e.g. predators, competitors, pond desiccation). However, environmental perturbations due to human influences are *additional* stresses that may disrupt homeostasis and lead to injury. Thus, it is important to examine multiple potential factors (both natural and anthropogenic) simultaneously when attempting to determine causes for amphibian declines.

Evidence suggests that ultraviolet-B (UV-B) radiation has the potential to decrease amphibian hatching success (Lizana and Pedraza, 1998) and increase embryonic mortality (Hakkinen et al., 2001). In addition, UV-B can have sublethal effects on amphibians by causing deformities (Blaustein et al., 1997; Ankley et al., 2000), altering behavior (Belden et al., 2000; Blaustein et al., 2000), and slowing growth and development (Pahkala et al., 2000, 2001; Smith et al., 2000). UV-B radiation has been implicated in causing specific declines (Broomhall et al., 2000). It is, however, important to note that there are differences among species in their sensitivity to UV-B (Blaustein et al., 1998) and that some species are affected very little or not at all (Licht and Grant, 1997; Corn, 1998).

There is also mounting evidence that contaminants are harmful to amphibians at environmentally relevant concentrations (Sparling et al., 2000; Davidson et al.,

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2001). Many amphibian species breed opportunistically in a range of aquatic habitats, some of which are embedded in agricultural or industrial landscapes; consequently, their larvae may be exposed to environmental contaminants at some time during development. For example, carbaryl (1-naphthyl-*N*-methylcarbamate) is an acetylcholinesterase-inhibiting insecticide, widely used throughout the US and Canada. It has been found in field concentrations up to 4.8 mg/l immediately following application (Norris et al., 1983; Peterson et al., 1994). It can contaminate aquatic environments via direct application, drift from spraying or in runoff from agricultural or urban application sites. Carbaryl has been the subject of numerous laboratory (Bridges, 1999a,b, 2000) and field studies (Boone and Semlitsch 2001, 2002; Boone et al., 2001) and can, therefore, serve as a model insecticide. The toxicity of carbaryl increases with temperature (Boone and Bridges, 1999) and with UV-B intensity. Zaga et al. (1998) found that the toxicity of carbaryl to tadpoles increased by 10-fold in the presence of UV-B in the laboratory at an intensity of 1.5% of ambient solar UV-B. Therefore, we predicted that this pesticide may be more potent in the field, under natural light, than in the laboratory.

Our objective was to examine the effects of the insecticide carbaryl (a man-made stressor) on the survival, growth, and development of southern leopard frog (*Rana sphenoccephala*) tadpoles under varying UV-B (a natural stressor) intensities. We conducted our study in cattle tanks because they present more realistic conditions than in laboratory studies, yet are more easily controlled than natural ponds (Rowe and Dunson, 1994).

## 2. Materials and methods

Our experiment was designed as a complete factorial, with three UV-B intensities, two insecticide treatments, and three replicates. Artificial ponds were created using eighteen polyethylene cattle tanks (1.85 m diameter; 1480 l volume), which were filled with 1000 l of well water and 1 kg of leaf litter on 16 March 2001. Each pond was inoculated three times with plankton from natural ponds over three weeks by adding 250 ml of whole water samples from established ponds. After filling, the artificial ponds were covered with screen-mesh lids to prevent colonization from other anurans and predatory insects. Forty days after the ponds were set up, the screen-mesh lids were replaced with one of three UV-filtering lids. Each lid was constructed by affixing the filter onto a  $\sim 2$  m<sup>2</sup> wooden frame. The frame was wrapped with either a double layer of Saran<sup>®</sup> plastic wrap (polyvinylidene chloride; high UV-B treatment), a single sheet of mylar-D (0.005 inch thickness, Cope Plastics, Inc., St. Louis, Mo.; medium UV-B treatment),

or with one layer of polycarbonate plastic (0.030 inch thickness, Cope Plastics, Inc. St. Louis, Mo.; low UV-B treatment). On the day prior to the introduction of the tadpoles into the tanks, the UV-B intensity was measured under each lid type at various depths of a single pond using a scanning spectroradiometer (Optronics Laboratories, model 754, Orlando, FL) equipped with a 15 cm diameter underwater integrating sphere and quartz fiber optics cable (Table 1).

On 12 April 2001, southern leopard frog (*Rana sphenoccephala*) tadpoles were collected from three egg masses in an ephemeral pond near Fayette, Missouri (Howard County). Eggs were allowed to hatch in aquaria, tadpoles were fed ad libitum TetraMin fish flakes, and aquaria water was changed every third day. On 25 April 2001, 45 tadpoles were added to each pond. The next day Sevin<sup>®</sup> (22.5% carbaryl) was added to the ponds assigned to hold a chemical treatment. We mixed 22.2 g Sevin<sup>®</sup> with 5 l of pond water and sprinkled this into each tank with a watering can to reach a target concentration of 5.0 mg/l (approximating post-application, environmentally expected concentrations; Norris et al., 1983; Peterson et al., 1994). In ponds receiving no chemical, 5 l of pond water was sprinkled over the surface as a control. Dissolved oxygen (range 2.5–4.9 mg/l), pH (range 6.9–7.1) and temperature (range 16.4–16.9 °C) were measured after carbaryl was added. One hour after application, a composite sample comprised of water from three ponds exposed to carbaryl (one from each UV filter type) was collected and refrigerated until sent for carbaryl analysis (Mississippi State Chemical Laboratory) by high pressure liquid chromatography. Forty-eight hours after chemical application, composite samples were collected from three ponds, exposed to carbaryl for each UV filter type (i.e. three carbaryl samples) and sent for analysis. Water samples were taken for chlorophyll analysis from each pond before (26 April 2001), then after (12 June 2001) carbaryl application to estimate algal food resources available for tadpoles. To do this, 6-l composite water samples were taken from each pond. A subsample of 100 ml was filtered and placed into 15 ml of neutralized 90% acetone in the dark at 5 °C for 24 h prior to fluorometry (Greenburg et al., 1992). Temperature in three ponds, each with a different UV-B treatment, was periodically monitored with maximum/minimum thermometers and never differed from one another by more than 2 °C.

The first metamorphs were collected on 29 May 2001. At this time lids were removed from all ponds to allow the collection of metamorphs (characterized as having at least one forelimb emerged; stage 42; Gosner, 1960) because any UV effects would have been manifested by this time and any threats from invertebrate predator colonization were low. Metamorphs were collected daily and held individually in the laboratory until tail resorption, at which time they were weighed to the nearest

Table 1

The intensity of ultraviolet-B radiation (UVB; top number), UV-A (middle number), and total visible light (bottom number) in  $\mu\text{W}/\text{cm}^2$  measured at various depths within a single cattle tank for each treatment<sup>a</sup>

Cover type	Ambient	Subsurface	10 cm depth	Bottom
No cover (for reference)	245.0	56.80	3.97	0.006
	5726	1638	401	22.9
	53,867	30,249	18,473	13,671
Plastic wrap (high UV-B)	233.0	46.00	1.50	0.004
	n/a	1583	335	18.3
	n/a	18,969	14,216	7488
Mylar D (medium UV-B)	109.0	27.15	0.12	0.005
	2643	1253	272	17.8
	28,566	18,015	14,301	11,982
Polycarbonate (low UV-B)	27.3	0.54	0.004	0.001
	402	162	32.8	3.43
	37,055	21,108	16,414	12,252

<sup>a</sup> Ambient readings were recorded just above the surface of the water, and subsurface readings just below the surface. UV-B value is the intensity averaged from 280 to 320 nm, UV-A value is the mean from 320 to 400 nm, and visible light is the mean from 400 to 700 nm.

milligram. The experiment was ended on 26 June 2001 because most tadpoles (~98%) had either metamorphosed or died.

The effects of UV-B intensity and the presence of carbaryl on survival to and mass at metamorphosis and the duration of the larval period were analyzed using analysis of variance (ANOVA). Mass at metamorphosis and the duration of the larval period often depend on the density of tadpoles within an environment (Wilbur, 1977), and these traits can be affected by differential survival among treatments. Therefore, we used survival as a covariate in initial analyses, but removed it from models in which it was found to be non-significant. Average values for each pond were derived and used in all analyses. Repeated-measures ANOVA was used to determine whether there were differences in chlorophyll concentrations among ponds attributable to UV intensity or the presence of carbaryl at the two sampling dates. All values were log transformed to increase normality (Shapiro–Wilk > 0.05).

### 3. Results

Our high UV-B treatment provided UV irradiance similar to ambient (unfiltered) conditions, medium UV-B filters removed half the ambient UV-B, and our low UV-B treatment provided only about 10% of ambient UV-B irradiance into the cattle tanks (Table 1). Because the measurements represent means for three replicate readings at each depth for each lid type and were all taken within a single pond, we did not determine the range of variation among the cattle tanks. The carbaryl concentration in each pond type dropped from a measured concentration of 4.1 mg/l at 1 h post-application

to an average of 0.643 mg/l within 48 h of application (low UV-B = 0.690 mg/l; med UV-B = 0.430 mg/l; high UV-B = 0.810 mg/l) indicating that breakdown was rapid and similar among UV-B treatments.

UV-B intensity significantly increased survival to metamorphosis (Fig. 1) but did not affect mass at metamorphosis or the duration of the larval period (Table 2). The presence of carbaryl significantly increased mass at metamorphosis (Fig. 2; Table 2), but had no effect on the duration of the larval period. Tadpoles in tanks containing carbaryl were 20% larger than those in tanks without carbaryl. Chlorophyll content in the ponds increased over time (Wilks' Lambda  $F_{1,12} = 25.09$ ;  $P = 0.0003$ ). At the second sampling date, the chlorophyll concentration in the ponds with carbaryl was 347% greater than in control ponds ( $F_{1,12} = 10.94$ ;  $P = 0.0063$ ; Fig. 2).

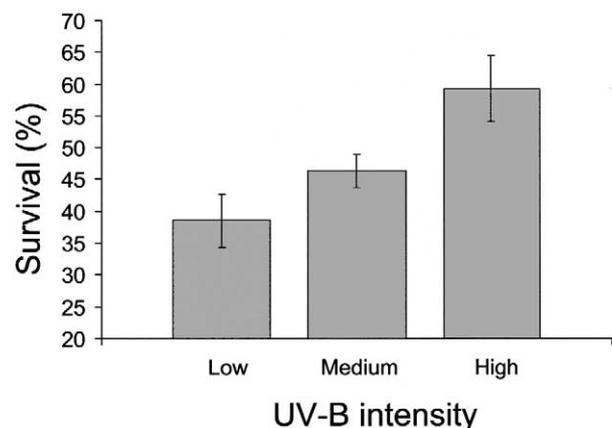


Fig. 1. Percent of tadpoles surviving until metamorphosis in each of the UV-B treatments. Vertical lines represent  $\pm 1$  SE.

Table 2

Univariate analyses of variance on the effects of UV-B and carbaryl on the survival, the duration of the larval period, and mass at metamorphosis of tadpoles of *Rana sphenocephala*. Type III mean squares are reported

Response variable	Source	df	MS	F	P
<i>Survival</i>					
	UV-B	2	0.0803	6.55	0.0119
	Carbaryl	1	0.0004	0.04	0.8484
	UV-B×Carbaryl	2	0.0266	1.08	0.3693
	Error	12	0.1472		
	Total	17	0.3160		
<i>Length of larval period</i>					
	Survival	1	0.0032	0.95	0.3499
	UV-B	2	0.0045	1.33	0.3045
	Carbaryl	1	0.0020	0.59	0.4586
	UV-B×Carbaryl	2	0.0030	0.44	0.6523
	Error	11	0.0374		
	Total	17	0.0563		
<i>Metamorph mass</i>					
	Survival	1	0.0006	0.01	0.9159
	UV-B	2	0.0351	0.63	0.5496
	Carbaryl	1	0.2706	4.87	0.0494
	UV-B×Carbaryl	2	0.0429	0.77	0.4851
	Error	11	0.6106		
	Total	17	0.9937		

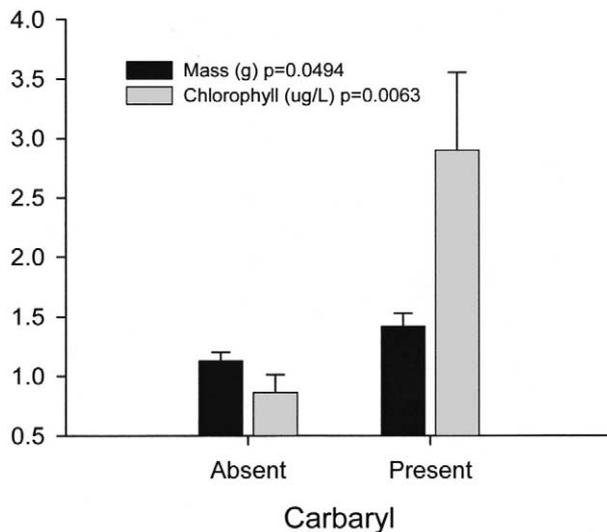


Fig. 2. Mass at metamorphosis (black bars) and chlorophyll content of ponds (gray bars) in the presence and absence of carbaryl. Vertical lines represent  $\pm 1$  SE.

#### 4. Discussion

Many studies have documented either decreased survival in the presence of UV-B (Broomhall et al., 2000), or no effect on survival (Smith et al., 2000). Aside from our study, the only research we are aware of that demonstrates an increase in larval survival in the presence of ambient UV-B is Bridges et al. (in preparation). Most previous

UV-B studies have been conducted on embryos (e.g. Blaustein et al., 1998; but see Pahkala et al., 2001). The tadpoles used in our study had hatched two weeks prior to being introduced into the ponds, so tadpoles may be less sensitive to UV-B exposure than embryos. Crump et al. (1999) observed that larger tadpoles were more sensitive to UV-B than embryos, and hypothesized that embryos have protection (e.g. egg capsules, high dermal melanin) that tadpoles do not. However, tadpoles in their study were confined to a shallow depth (i.e. 4.5 cm) within the pond. In our study, tadpoles were able to swim into the leaf litter, and to depths where little UV-B penetrates (Table 1). Before tadpoles began to metamorphose, few individuals were seen near the surface of the water in any treatment. It is possible that our filters eliminated a range of wavelengths critical for vital functions that resulted in reduced survival among tadpoles in the low UV-B treatment (e.g. vitamin D production in humans). The increase in survival in the high UV-B treatment was not attributable to greater algal resources bolstered by additional UV-B radiation as there were no significant differences related to chlorophyll and UV-B intensity. However, sampling at only two dates may have prevented us from detecting differences during critical stages of development.

Unless tadpoles inhabit very clear or shallow water, it is unlikely that they will be exposed to UV-B levels that are as high as some of those tested in previous studies. In the cattle tanks, as in most other naturally occurring ponds (Hurtubise et al., 1998), most of the UV-B below 10 cm was filtered out. Also, tadpoles were generally found in the leaf litter at the bottom of the tank. In addition to the shading provided by the leaves, the dissolved organic carbon (DOC) present in the pond likely served to shield tadpoles from harmful levels of UV-B (Morris et al., 1995). Possibly for these reasons, UV-B radiation did not increase the toxicity of carbaryl as we had expected. DOC concentration may also play a role in the fate of chemicals such as carbaryl by limiting the radiation necessary for the breakdown to more harmful compounds. Furthermore, compounds such as humic and tannic acids may also bind to the chemical making it less available to the biota. Overestimating the importance of UV-B can also occur when developing animals are maintained at a water depth more shallow than what would normally occur (e.g. Ovaska et al., 1997; Pahkala et al., 2001). We recommend directly measuring UV-B at varying depths in the natural environment to understand the natural range of UV-B intensities to which developing larvae are exposed. We did not directly measure DOC, but the water in all ponds was a distinctive tea-color, indicating that the deciduous leaves in the ponds had leached out tannins producing elevated DOC concentration.

Tadpoles reared in tanks that had been dosed with carbaryl were larger at metamorphosis than tadpoles in

control tanks, regardless of UV-B intensity. Chlorophyll concentration was greater in the ponds dosed with carbaryl, indicating that anurans had more food resources. This phenomenon has been observed in other studies using carbaryl (Boone and Semlitsch, 2002). Carbaryl at very low concentrations kills zooplankton (Hanazato and Yasuno, 1987, 1990), which often results in an algal bloom providing more food for tadpoles for growth (Mills, 2002). There are a number of benefits for amphibian larvae achieving a large mass at metamorphosis, including greater overwintering success, greater survival to first reproduction, and earlier reproduction (Smith, 1987; Semlitsch et al., 1988). Further, larger females can carry more eggs, and larger males often gain access to a greater number of females during breeding, leading to increased reproductive success (Berven, 1982).

Because of the advantages of increased mass at metamorphosis, it appears as if the addition of carbaryl had a positive impact on the amphibians in the community. However, Rapport et al. (1985) suggest that any alteration in the primary productivity in a system can signify ecosystem dysfunction. Clearly, the inhibition of the zooplankton population exposed to carbaryl is severe (Mills, 2002; MDB personal observation) and could lead to decreased species diversity and alter food-web dynamics in an unpredictable manner. For example, while eliminating zooplankton may be beneficial for herbivorous tadpoles, the consequences of this shift is detrimental to carnivorous amphibians (e.g. salamander larvae; Boone and James, in press) and severely alters community structure. Furthermore, an increase in primary productivity could also lead to algal blooms and eutrophication, which would eventually harm amphibian populations.

Our study emphasizes the importance of more realistic field studies when examining the effects of UV-B radiation on amphibians. Tadpoles frequently inhabit environments having DOC and are able to behaviorally avoid high UV intensity (Belden et al., 2000). Most studies have not identified the range of ambient field irradiance levels. Therefore, doses resulting when even 95% of the UV-B radiation is filtered out in a laboratory experiment may expose certain organisms to intensities of radiation that are unrealistic in a natural setting. This is especially important given the recent attention UV-B has received as to possibly attributing to declining amphibian populations.

#### Acknowledgements

We thank S. James, S. Saura, and J. Wells for their technical assistance. R. Semlitsch and E. Little provided us with necessary space and materials. This manuscript was improved through the thoughtful comments of

R. Calfee, E. Little, S. James, R. Semlitsch, and two anonymous reviewers.

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