Variation in Pesticide Tolerance of Tadpoles among and within Species of Ranidae and Patterns of Amphibian Decline

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Abstract: *There is significant variation among and within amphibian species with respect to reports of population decline; declining species are often found in environments that are physiographically similar to environments where the same species is thriving. Because variability exists among organisms in their sensitivity to environmental stressors, it is important to determine the degree of this variation when undertaking conservation efforts. We conducted both lethal (time-to-death) and sublethal (activity change) assays to determine the degree of variation in the sensitivity of tadpoles to a pesticide, carbaryl, at three hierarchical levels: among ranid species, among several populations of a single ranid species (*Rana sphenocephala*), and within populations of* R. sphenocephala. *We observed significant variation in time to death among the nine ranid species and among the 10* R. sphenocephala *populations we tested. Four out of eight* R. sphenocephala *populations exhibited significantly different times to death among families. The magnitude of the activity change in response to exposure to sublethal carbaryl levels was significantly different among species and within* R. sphenocephala *populations. Chemical contamination, at lethal or sublethal levels, can alter natural regulatory processes such as juvenile recruitment in amphibian populations and should be considered a contributing cause of declines in amphibian populations.*

Variación de la Tolerancia a Pesticidas en Renacuajos entre y dentro de Especies de la Familia Ranidae y Patrones de Disminución de Anfibios

Resumen: *Existe una variación significativa entre y dentro de especies de anfibios con respecto a los reportes de disminuciones poblacionales; las especies en disminución son frecuentemente encontradas en ambientes que son fisiográficamente similares a ambientes donde las mismas especies están prosperando. Debido a que la variabilidad existe entre organismos en lo referente a su sensibilidad a estresores ambientales, es importante determinar el grado de esta variación cuando se lleven a cabo esfuerzos de conservación. Nosotros llevamos a cabo ensayos tanto letales (tiempo de muerte) como subletales (cambios de actividad) para determinar el grado de variación en la sensibilidad de renacuajos a un pesticida, carbaryl, en tres niveles jerárquicos: entre especies de ránidos, entre diversas poblaciones de una sola especie de ránido (*Rana sphenocephala*) y dentro de poblaciones de* R. sphenocephala. *Observamos diferencias significativas en tiempo de muerte entre las nueve especies de ránidos y entre las 10 poblaciones de* R. sphenocephala *evaluadas. Cuatro de ocho poblaciones de* R. sphenocephala *exhibieron tiempos de muerte significativamente diferentes entre familias. La magnitud del cambio de actividad al ser expuestas a niveles subletales de carbaryl fue significativamente diferente entre especies y dentro de poblaciones de* R. sphenocephala. *La contaminación química, a niveles letales y subletales, puede alterar los procesos reguladores naturales (por ejemplo el reclutamiento de juveniles) en poblaciones de anfibios y debería ser considerado como una causa que contribuye a la disminución de poblaciones de anfibios.*

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Introduction

A growing body of evidence suggests that a number of amphibian populations have declined in recent years (Barinaga 1990; Blaustein & Wake 1990; Wake 1998). The cause of these declines has been difficult to establish because in some instances only a single species is declining whereas sympatric species are thriving (e.g., McAllister et al. 1994). Similar variation can also be observed within a single species at the population level: there are often instances when some populations of a particular species are declining and others remain unaffected (e.g., *Rana pipiens* [Corn & Fogleman 1984], *Rana muscosa* [Bradford 1991]). Because not all populations or species have been affected, it is important for conservationists to identify causes for the variability among populations whose environments appear physiographically similar. Research efforts should therefore be focused on addressing the question of what makes some species and populations vulnerable and others resistant to declines. Our primary objective was to determine the degree of variation present in amphibians with respect to their responses to pesticide exposure. In doing this, it may be possible to establish potential patterns of vulnerability among and within species.

Chemical contaminants are not homogeneous stressors because their presence in the environment varies over small temporal and spatial scales. Variation in the response of organisms to a contaminant can thus arise from the differential exposure that results from this variability in the presence of the contaminant. Variation in response may also be related to genetic differences among or within species (Hecnar 1995; Berrill et al. 1994). Populations with little or no heritable genetic variability in tolerance may be more susceptible to decline and local extinction because they cannot adapt to the presence of environmental stressors. In the long term, a high degree of heritable genetic variation can determine the persistence of a population in a changing environment (Lande & Shannon 1996), such as in the case of increasing levels of contamination that are occurring annually (Connell & Miller 1984).

Inter- and intraspecific variation has been observed across many taxa with respect to resistance to natural environmental stressors, including desiccation (Arad et al. 1993; Asami 1993), salinity (Allen et al. 1994; Kandl & Thompson 1996), herbivory (Byington et al. 1994), and anthropogenic stressors such as pH and chemical contaminants (Blanck 1984; Naylor et al. 1990; Berrill et al. 1994). Variation in response to environmental stressors has also been observed among families within a single population (Clark & LaZerte 1987; Byington et al. 1994).

To assess the degree of variation in response to an anthropogenic stressor among and within species of frogs in the family Ranidae, we examined the variation in tolerance of tadpoles to the pesticide carbaryl. We examined variation in a hierarchical fashion to identify where variation was greatest: among nine ranid species; among populations within a single species, the southern leopard frog (*Rana sphenocephala*); and within populations of *R. sphenocephala* (i.e., among families). We used ranid frog species because many declines in the United States have occurred within this family (Hayes & Jennings 1986). Furthermore, although data are available for many ranid species regarding their sensitivity to chemicals, it seems that no single species in this family is consistently more tolerant than another (Table 1). Because three of the species we examined are declining in portions of their range, we were able to test an important hypothesis of amphibian conservation efforts: species in decline are more sensitive to environmental stressors such as chemical contamination, which may partially explain observed patterns of decline.

Methods

Species and Populations Sampled

We conducted tests over 3 years (1996–1998); frogs used for species comparisons were collected in 1996 and 1997, and frogs for the within-species tests were collected in 1996–1998. No population was sampled more than 1 year. We selected species based on their presence within our own region, the midwestern United States, and on the availability of volunteers to collect and ship samples from other regions of the United States and Canada. For the 1996 comparison the following species were collected: *Rana aurora*, Santa Cruz County, California; *R. boylii*, Trinity County, California; *R. pretiosa*, Thurston County, Oregon; *R. sylvatica*, Edmonton, Alberta, Canada; and *R. palustris*, Boone County, Missouri. In 1997 we collected *R. blairi* from Cole County, Missouri; *R. clamitans* from Boone County, Missouri; and *R. areolata* from Williamson County, Illinois. To increase the probability that each test sample included a representative sample of genetic variation within the population, at least three egg masses from each species and from each population were used in each experiment. *Rana sphenocephala* tadpoles were collected during all 3 years from several locations.

To examine variation in response among populations within *R. sphenocephala*, 10 populations were sampled. *Rana sphenocephala* is widely distributed throughout the southeastern United States, inhabiting many different habitat types, including those in disturbed and agricultural landscapes. Across its range, this species may encounter a variety of environmental stressors and is therefore an excellent species in which to examine the extent of variation in response. Tadpoles from separate egg masses from each population were tested to determine within-population (i.e., among-family) variation, ex-

Table 1. Comparison of sensitivity among tadpoles of ranid species in the United States to various contaminants.

Contaminant	*Species (in descending order of sensitivity)*[a]	*Reference*
Ammonium nitrate	*R. pipiens* *R. clamitans*	Hecnar 1995
Diazinon	*R. clamitans* *R. pipiens*	Harris et al. 1998
Dieldrin	*R. catesbieana* *R. pipiens*	Schuytema et al. 1991
Endrin	*R. catesbieana* *R. sphenocephala* *R. sylvatica*	Hall & Swineford 1981
Fenitrothion	*R. catesbieana* (A) *R. clamitans* (A) *R. sylvatica* (B) *R. pipiens* (B)	Berrill et al. 1995
Fenitrothion, tricopyr, hexazinone	*R. catesbieana* *R. clamitans* *R. pipiens*	Berrill et al. 1994
Mercuric chloride	*R. sphenocephala* *R. clamitans* *R. catesbieana*	McCrary & Heagler 1997
Mexacarbate	*R. sylvatica* *R. clamitans*	Rick & Price 1974[b]
pH	*R. clamitans* (C) *R. sphenocephala* (C) *R. sylvatica* *R. palustris*	Freda 1986
Toxaphene	*R. catesbieana* *R. sphenocephala* *R. sylvatica*	Hall & Swineford 1981

[a] *Within studies, species with the same letter do not differ significantly from one another. When no letters are present, all species differ from one another.*
[b] *As reported by Power et al. (1989).*

cept for those from Illinois and Texas, which were each pooled because of sampling constraints. In 1996, we tested seven egg masses from a single population in Aiken County, South Carolina. The origin of the 1997 populations used in this experiment and the number of egg masses collected were as follows: two *R. sphenocephala* populations from Boone County, Missouri (abbreviated Mo2, $n = 3$; Mo3, $n = 3$), and one population from each of the following places: Williamson County, Illinois (IL, $n = 3$); Lafayette County, Mississippi (Ms, $n = 5$); Cole County, Missouri (Mo1; $n = 18$); Webster County, Missouri (Mo4, $n = 2$); and Wilson County, Tennessee (Tn, $n = 3$). In 1998, egg masses were collected from Montague County, Texas (Tx, $n = 3$), and from King and Queen County, Virginia (Va, $n = 4$). The exact location and habitat parameters of all collection sites and of species and populations tested are reported by Bridges (1999*a*).

When necessary, samples were shipped via express delivery service at the egg or early tadpole stage (≤ stage 25; Gosner 1960) in insulated coolers packed with ice containers. Upon receipt, we maintained each tadpole group in a 30-L stainless steel tank with a constant flow of well water (22 ± 1° C). Tadpoles were fed fish flakes ad libitum. Although tadpole sizes differed slightly among groups, all tadpoles were tested at uniform stages (stages 25–27; Gosner 1960).

Carbaryl

Carbaryl (1-naphthyl-N-methylcarbamate) is one of the most commonly used broad-spectrum insecticides in the United States, currently applied to crops such as soybeans at a rate of 2500 to 3500 metric tons per year (U.S. Environmental Protection Agency 1992). Carbaryl is of low toxicity to vertebrates and is consequently the active ingredient in many common garden insecticides and flea powders. An advantage of carbaryl is that it breaks down quickly after application (approximately 10 days; Peterson et al. 1994), which minimizes the risk of exposure to nontarget organisms such as amphibians. Aquatic habitats can become contaminated with carbaryl directly from drift from aerial spraying and indirectly through runoff from gardens or agricultural fields. Carbaryl is an acetylcholinesterase-inhibiting compound with a mode of action similar to that of many currently used pesticides (e.g., carbamates, organophosphates). Therefore, carbaryl can

serve as a model chemical with which to examine the effects of many pesticides on amphibians and amphibian larvae.

Each year, we created a new stock solution of carbaryl by dissolving 6.018 g of powdered carbaryl (99.8% purity) into 100 mL of technical grade acetone. Between uses the stock solution was sealed and refrigerated. Because of the expense involved in chemically analyzing carbaryl, only stock solutions were analyzed (with high-pressure liquid chromatography) to determine measured concentrations. Consequently, all experimental concentrations were nominal but were based on measured stock concentration, which varied among years in relation to nominal concentrations as follows: 104% in 1996, 109% in 1997, and 95% in 1998.

Time-to-Death Assays

The most common toxicological test used to determine sensitivity to a contaminant is the LC50 (Rand et al. 1995). An LC50 is the concentration at which 50% of a test population dies when exposed to several concentrations of a chemical. An LC50 test often cannot be used on species whose numbers are limited because a large number of animals are required ($n \geq 200$). In our experiments, we used time-to-death assays, which examine mortality at a single concentration across time (i.e., rate of mortality). Time-to-death studies are useful because they accurately resolve which individuals are most tolerant but use a minimum number of individuals ($n = 10$–20). Furthermore, it has been demonstrated that time to death is correlated with 24- and 48-hour LC50s for tadpoles ($r = 0.88$, $p = 0.0090$, and $r = 0.83$, $p = 0.0200$, respectively; Bridges 1999*a*), indicating that time-to-death assays serve as an acceptable surrogate for LC50s when the relative tolerance of organisms is being measured.

Time to death was measured by placing individual tadpoles in 250-mL glass beakers containing 200 mL of a 30 mg/L carbaryl solution created by adding 0.1 mL of carbaryl stock solution to 200 mL of well water. Although 30 mg/L is greater than expected field concentrations (≤4.8 mg/L; Norris et al. 1983), it is only slightly higher than the average 24-hour LC50 values for a number of ranid species (e.g., *R. clamitans*, 22.55 mg/L; Boone & Bridges 1999). By exposing tadpoles to such a high concentration, we were able to elicit mortality (an experimental endpoint) within 48–60 hours. Additional beakers filled with 200 mL of well water and containing 0.1 mL acetone served as solvent controls. Because preliminary studies indicated that tadpole mortality and activity did not differ between water and solvent controls, only solvent controls were used. All beakers were held in a water bath and maintained at 22 ± 1° C. Tadpoles were not fed during exposure. There was no mortality in any control. Each family was replicated 10 times, except in three cases in which fewer individuals were available. Mortality was determined at 3, 6, 9, 12, 18, 24, 36, 48, and 60 hours after the beginning of a test and was defined as the absence of all movement after repeated prodding. After each tadpole died, it was weighed (wet weight) to the nearest 0.1 mg.

Activity Changes

Decreased activity levels over time can attenuate tadpole growth and development because of decreased food intake. We monitored spontaneous swimming activity to determine whether tadpoles were affected differentially by sublethal levels of carbaryl. The sublethal concentration of carbaryl we selected (2.5 mg/L) is slightly lower than the concentration expected in the field after application (Peterson et al. 1994). Individual tadpoles were placed in 250-mL glass beakers containing 200 mL of a 2.5-mg/L carbaryl solution, which we created before adding tadpoles by mixing 0.08 mL of the carbaryl stock solution to 200 mL of well water. The controls in the time-to-death experiment served as controls for this experiment as well because both tests were performed simultaneously and in the same water baths. Each family was replicated 10 times (i.e., 1 tadpole = 1 replicate), except in three cases in which fewer individuals were available. Tadpoles from South Carolina were not tested. Activity was monitored after 24 hours of exposure. Each tadpole was observed in succession for spontaneous activity—presence or absence of tail movements—for 5 seconds per observation once every 5 minutes. A total of 20 observations per tadpole were recorded by a single observer. By comparing exposed and control tadpoles, we were able to calculate the percent decrease in activity for each group tested.

Statistical Analyses

Standardized procedures used across the 3 years of this study should allow for reasonable comparisons of values among years. Data for species and populations were statistically confounded with year, however, because different species and populations were sampled in each year. Therefore, if there were significant differences ($p \leq 0.05$) among years according to one-way analysis of variance (ANOVA), we analyzed data by sampling year. When between-year sampling differences were not significant, we pooled data across years. To determine pairwise differences, we used least-significant-difference multiple comparison tests. In all ANOVAs, Type III SS were used to determine whether an effect was significant because of unequal sample sizes (Zar 1984).

Time-to-death data for species were pooled across years (i.e., there were no significant differences be-

tween the 2 years; $F_{1,121} = 1.26$, $p = 0.3592$) and were analyzed by ANOVA. Tadpole mass was used initially as a covariate to control for the effect of body size on the variation in time to death, but this effect was never significant and was subsequently removed from all models. To include *R. sphenocephala* in the among-species analyses, it was necessary to derive a single sample population by randomly choosing 20 representative individuals from the larger sample, which included over 400 individuals from several populations. Using this random sample allowed greater design balance, and because the responses of these individuals were normally distributed, we believe this sample to be representative of the species.

Times to death for populations within *R. sphenocephala* differed significantly among the 3 years ($F_{2,398} = 12.33$; $p < 0.0001$), so they were analyzed according to year. Time-to-death differences among populations and between families within populations of *R. sphenocephala* were analyzed by a nested analysis of covariance, with tadpole mass as a covariate and nesting families within populations. Also, because families within populations represent a random sample of all available families, we considered family a random effect, whereas we designated population as a fixed effect in the statistical models. Differences among families within populations were determined with a separate ANOVA for each population. Texas and Illinois populations could not be analyzed in this manner because families in each of these populations were pooled.

The proportion of time spent active was calculated for each tadpole by dividing the number of observation periods spent active by the total number of observation periods (i.e., 20). All activity data were arcsine square-root transformed (Zar 1984) and normally distributed as determined by a Shapiro-Wilk test. Data for comparing species were pooled across years (i.e., there were no significant differences among years; $F_{1,259} = 1.58$, $p = 0.2104$) and analyzed by ANOVA to determine the effects on tadpole activity of carbaryl treatment, species, and the interaction of treatment and species. Data within species were analyzed according to year (i.e., there were significant differences among years; $F_{1,594} = 13.66$, $p = 0.0002$) with a nested ANOVA to determine the effects on tadpole activity of carbaryl treatment, population, family within population, and the interaction these effects. Family differences within population in activity, treatment, and their interaction were analyzed by means of a separate ANOVA for each population. As noted for the time-to-death analysis, Texas and Illinois populations could not be analyzed because families in each of these populations were pooled.

To determine whether groups that were tolerant with respect to TTD (i.e., high times) showed the lowest decrease in activity, we conducted a Pearson's correlation analysis between time to death and the percent decrease in activity for each group tested.

Results

Among Species

There were significant differences among the nine ranid species in time to death ($F_{8,162} = 10.11$; $p < 0.0001$; Fig. 1). Mean time to death varied from 5 to 34 hours (Fig. 1). Pairwise comparisons indicated that two of the three western species (*R. pretiosa* and *R. aurora*) demonstrated significantly greater tolerance than all other species, whereas *R. sylvatica* was significantly more sensitive than all other species.

There were significant differences among species with respect to their overall activity levels ($F_{7,245} = 4.27$, $p < 0.0002$). Also, all of the eight species tested showed a decline in activity level due to carbaryl (average decline = 18.5% ± 0.8% SE; $F_{1,245} = 140.44$, $p < 0.0001$). There was, however, no significant interaction of treatment and species ($F_{7,245} = 1.01$, $p = 0.4228$). Because a statistically significant interaction between species and treatment was necessary to demonstrate that the magnitude of the decreased activity was dependent upon tadpole species, the lack of an interaction indicated that all species were equally sensitive to carbaryl exposure.

Among *Rana sphenocephala* Populations

Time to death significantly differed among *R. sphenocephala* populations in each of the 3 years (Table 2; Fig. 2). Populations from Texas, Mississippi, South Carolina, and one from Missouri were most tolerant, whereas populations from Virginia and Illinois and three from Missouri were most sensitive. Activity levels of the various

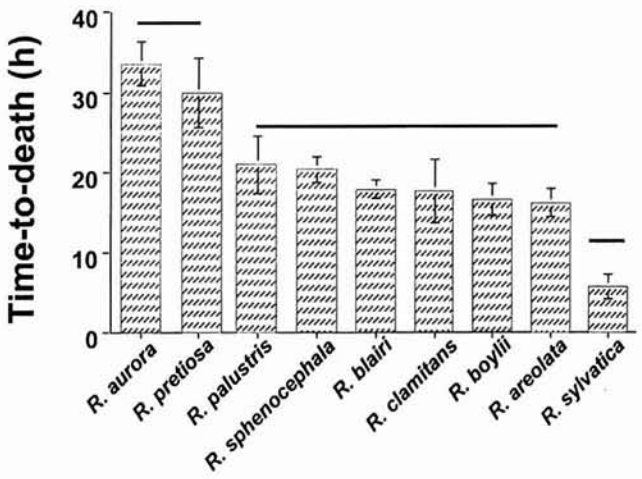

Figure 1. Time to death (hours) after exposure to carbaryl for each of the nine ranid species tested. Horizontal lines group species that do not significantly differ from one another ($\alpha = 0.05$). Vertical lines on bars represent ±1 SE.

R. sphenocephala populations differed significantly from one another in 1997 but not in 1998 (Table 3). Carbaryl also significantly reduced the activity of *R. sphenocephala* tadpoles in 1997 and 1998. In each of the 2 years there was a significant population-by-treatment interaction, indicating that populations were differentially sensitive to carbaryl exposure (Fig. 3). Activity in three populations (e.g., Mo3, Va, Tn) decreased sharply in the presence of carbaryl, whereas others appeared unaffected (e.g., Mo1, Tx; Fig. 3).

Among Families within Populations of *Rana sphenocephala*

Although in 1996 and in 1998 there were no significant differences among the families within each population, there was significant variation in 1997 (Table 2). Within *R. sphenocephala* populations there were significant differences in time to death among families in four out of the eight populations tested (Table 4). Although carbaryl decreased the activity of all families within populations of *R. sphenocephala*, there were no significant family-by-treatment interactions (Table 3).

Correlations

There was a significant negative correlation between the percentage decrease in activity and time to death for eight *R. sphenocephala* populations ($r = -0.8936$, $p = 0.0012$).

Discussion

Among-Species Variation

Localized declines have been noted in all ranid species in the western United States (Corn & Fogleman 1984; Hayes & Jennings 1986; Bradford 1991; Fellers & Drost 1993; Bradford et al. 1994*a*, 1994*b*; Drost & Fellers 1996), including the three western U.S. species in our experiment: *R. pretiosa* (U.S. Fish and Wildlife Service 1993; McAllister et al. 1994), *R. aurora draytonii* (U.S. Fish and Wildlife Service 1996), and *R. boylii* (Corn

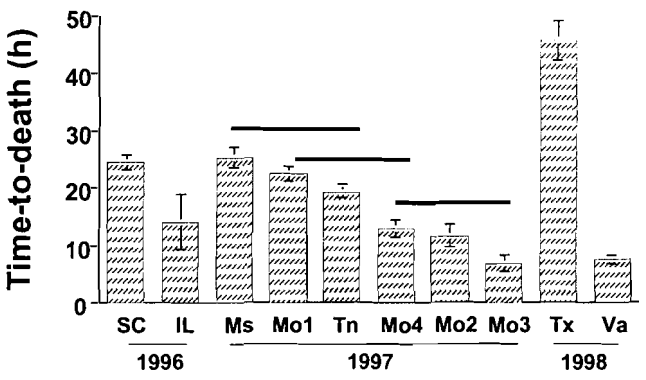

Figure 2. Time to death (hours) after exposure to carbaryl for each of the 10 Rana sphenocephala *populations tested (SC, South Carolina; IL, Illinois; Ms, Mississippi; Tn, Tennessee; Tx, Texas; Va, Virginia; Mo1-Mo4, four Missouri populations). In 1996 and 1998, both populations differed significantly from one another ($\alpha = 0.05$). In 1997, bars underneath the same horizontal line do not differ significantly from one another ($\alpha = 0.05$). Vertical lines on bars represent ±1 SE.*

Table 2. Differences in *Rana sphenocephala* tadpole time to death in response to carbaryl treatment among populations and families nested within populations over each of 3 years.

Year and source of variation	*df*	*Type III MS**	F	p
1996				
population	1	936.75	7.50	0.0078
family (population)	6	199.67	1.60	0.1600
error	71	124.84		
1997				
population	5	2098.91	23.66	0.0001
family (population)	21	488.85	5.06	0.0001
error	244	88.69		
1998				
population	1	11612.88	282.69	0.0001
family (population)	3	30.60	0.74	0.5310
error	45	41.08		

**Type III mean squares were used because of unequal sample sizes among sites.*

Table 3. *Rana sphenocephala* changes in activity among populations and among families within populations in response to carbaryl treatment during each of 3 years.

Year and source of variation	*df*	*Type III MS*	F	p
1996*				
carbaryl	1	0.25	4.12	0.0573
error	18	0.06		
1997				
population	5	1.09	38.17	0.0001
carbaryl	1	8.71	304.95	0.0001
family (population)	21	0.09	3.17	0.0001
population × carbaryl	5	0.31	10.86	0.0001
family × carbaryl × population	21	0.04	1.51	0.0691
error	443	0.02		
1998				
population	1	0.08	2.54	0.1144
carbaryl	1	0.45	13.13	0.0005
family (population)	3	0.03	0.89	0.4477
population × carbaryl	1	0.20	5.62	0.0199
family × carbaryl × population	3	0.02	0.61	0.6089
error	89	0.03		

**Only a single population was used in 1996.*

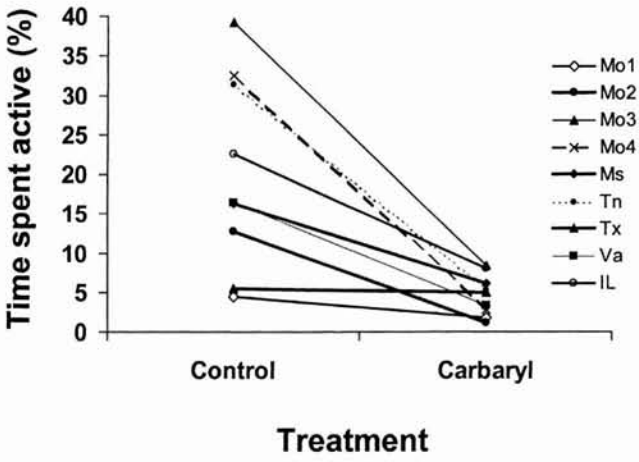

Figure 3. The percentage of time spent alive under normal (control) conditions and after 24 hours of carbaryl exposure. Each line represents the mean of a single population and the response in both treatments. See Fig. 2 for population abbreviations.

1994). Declines in these species may be more prevalent because they are more vulnerable to environmental stressors than are eastern species, which are not in decline. An increase in vulnerability could be caused by low genetic diversity resulting from small population sizes and restricted ranges, or it could be simply a function of evolutionary similarity. Further, the detrimental effects of environmental stressors could be exacerbated in these western species because gene flow among populations may be limited by fragmented distributions and long distances between populations, which make recolonization of declining or extirpated populations more difficult (Blaustein et al. 1994). If true, increased vulnerability to environmental stressors such as carbaryl or other pesticides in conjunction with limited dispersal among populations could contribute to observed amphibian declines.

Two of three western U.S. species we examined that have experienced the greatest declines, *R. pretiosa* and *R. aurora*, are those that were the most tolerant to carbaryl; the third, *R. boylii*, had an average tolerance. The theory of general-purpose genotypes (Lynch 1984) predicts that organisms with broad ranges such as eastern U.S. ranid species have greater tolerances, which are beneficial in dispersal, but this is not always true (Parker & Niklasson 1995). *Rana pretiosa* and *R. aurora* are more narrowly distributed than the other species we tested and may have become locally adapted to harsh environmental conditions and thus demonstrated higher tolerance to carbaryl in our experiment. Additional sampling of these two species from several populations would be necessary to determine whether the entire species demonstrates the same level of tolerance as the populations we sampled.

Any meaningful comparison of species must also consider that variability within a species could exceed and thus obscure the variability observed among species, as

Table 4. Results of separate analyses of variance on intrapopulation differences among *Rana sphenocephala* populations.[a]

Population[b], year tested	*df (numerator, denominator)*	*Type III MS (model, error)*	F	p
Mo1, 1996	3, 37	205.8, 63.1	3.26	0.0322
Mo2, 1997	3, 36	737.4, 106.0	6.96	0.0008
Mo3, 1997	2, 27	6.3, 56.3	0.11	0.8945
Mo4, 1997	1, 18	130.1, 40.1	3.25	0.0883
Ms, 1997	6, 63	883.0, 154.5	5.72	0.0001
SC, 1997	6, 62	199.6, 110.1	1.81	0.1112
Tn, 1998	6, 63	192.6, 55.9	3.45	0.0052
Va, 1998	3, 36	30.6, 22.3	1.33	0.2787

[a] *Results are from experiments on time to death in response to carbaryl treatment conducted on* R. sphenocephala *tadpoles from 1996 to 1998.*
[b] *Mo1-Mo4, four separate populations in Missouri: Ms, Mississippi population; SC, South Carolina population; Tn, Tennessee population; Va, Virginia population.*

we found with *R. sphenocephala*. Species with the greatest tolerance to overall stress may also exhibit high intraspecific variation in response to stress (Allen et al. 1994). For example, the two most tolerant species we examined (*R. pretiosa* and *R. aurora*) may have a high degree of intraspecific variation, and our sample populations may simply represent a tolerant population, whereas other populations may be more sensitive. This may explain why our third western U.S. species, *R. boylii*, was no more tolerant than any eastern U.S. species tested. Although it is possible that our *R. pretiosa* and *R. aurora* populations contained tolerant individuals, the populations from which these samples originated were not chosen because they constituted "pristine" or "contaminated" sites. Therefore, it is likely that these populations are representative and display mean responses rather than responses to either extreme (i.e., either more or less tolerant than the mean).

Mapping the sensitivity of ranid species on a phylogeny (e.g., Pytel 1986) would elucidate whether the responses we observed are correlated with evolutionary histories. Although a cursory comparison using our data suggests no taxonomic relationship in responses, our sampling design precludes us from drawing stronger conclusions. An effective comparison needs to include a greater number of species from a wider geographical area. Further, because most comparisons of ranid species are confounded with geographic distribution—most species occur only in the eastern or only in the western United States—it will be important to include more species inhabiting both regions (e.g., *R. pipiens*).

Within-Species Variation

We detected significant differences among populations in time to death for each of the 3 years we sampled *R.*

sphenocephala, indicating that a large degree of variation in response to chemical tolerance exists within populations throughout the range of this one species. It is unknown, however, whether these differences are attributable to local adaptation to an environmental stressor rather than to random effects or geographic variation associated with other traits. Local adaptation would require that (1) pesticides occur in the environment at concentrations low enough so as to not cause total mortality among frogs within a population, (2) pesticide tolerances within populations have a heritable genetic basis; (3) stressors persist in the environment between generations; and (4) fitness differences between tolerant and sensitive genotypes. Our sampling design precluded us from determining whether the within-species differences we observed had a genetic basis or whether collection sites had a history of carbaryl exposure, which might suggest adaptation to environmental stressors. Using half-sib breeding designs (Falconer & Mackay 1996), we determined that there was a significant amount of heritable variation in tolerance to carbaryl in the Mo1 population (heritability estimate = 0.28; Bridges & Semlitsch 2000). Thus, this population likely has the ability to adapt to environmental contaminants. Although an exact environmental history is not known for each *R. sphenocephala* population (for known details of collection sites, see Bridges 1999*a*), it is likely that each has been exposed historically to some pesticides. Therefore, it is possible that present tolerance levels result from previous, unrecorded contamination or other stress that affected general stress tolerance; hence, differences in sensitivity could be attributable to the lingering effects of a past environmental selective pressure of unknown intensity.

In our experiment, all tadpoles exposed to sublethal concentrations of carbaryl reduced their activity. Although there were no significant differences in the magnitude of activity change among the nine species examined, there were significant differences among the eight *R. sphenocephala* populations tested. The Texas population and one of the Missouri populations (Mo1) showed no significant decrease in activity, whereas the other seven populations demonstrated significant reductions (Fig. 3). The Texas and Mo1 populations were also the most tolerant with respect to time to death, demonstrating that their tolerance is exhibited in multiple responses related to fitness. Furthermore, these populations were the least active under control conditions, suggesting that activity level may be related to similar mechanisms for time-to-death resistance.

Concentrations of contaminants necessary to induce direct mortality are often much higher than any expected environmental concentration and therefore may be irrelevant to population declines in a natural setting. Consequently, examining the effects of sublethal concentrations of contaminants is more ecologically relevant. Although criteria for setting water-quality standards commonly include data from sublethal exposures, there are many cases in which legally acceptable concentrations have profound effects on organisms that often lead indirectly to mortality. For example, reduced activity can diminish feeding and lead to decreased growth and development, which can lengthen the larval period and reduce size at metamorphosis (e.g., Fioramonti et al. 1997). The length of the larval period and mass at metamorphosis are traits critical in determining an individual's fitness in terms of survival and future reproductive success. Amphibians that metamorphose at larger sizes and earlier in the season have a greater chance of surviving over winter and will reproduce at younger ages (Smith 1987; Semlitsch et al. 1988). Further, a short larval period is especially important to amphibian species breeding in temporary ponds, where any factor that lengthens the larval period, such as the presence of an environmental contaminant, can lead to indirect mortality due to desiccation. Sublethal levels of carbaryl decrease swimming performance (Bridges 1997), curtail tadpole predator avoidance behavior (Bridges 1999*b*), and consequently alter the dynamics between tadpoles and their predators (Bridges 1999*c*). As a result, low levels of a chemical such as carbaryl in the environment could cause a population to decline in numbers slowly over time.

Conclusions and Conservation Implications

Knowing which species are most sensitive to environmental stressors such as contaminants can elucidate which species are in danger of being decimated by such stressors. It is often difficult, however, to compare the sensitivity of species because of variation at the individual, family, and population levels and uncertainty about which stressors are ecologically relevant. Our study had the advantage that all experiments were conducted according to the same protocol and chemical stressor, thereby maximizing our ability to directly compare species. Although in some instances the responses of *Xenopus laevis* or *Rana pipiens* tadpoles, both common surrogates, to a toxicant are similar to that of natural populations of native species, there are often discrepancies (Mayer & Ellersieck 1986). Examining the responses of wild populations of native species, such as *R. sphenocephala*, is important in drawing conclusions about variation in the reported declines and in developing management plans to protect native species from future declines.

Conservation efforts for declining species must consider individual, family, population, and geographic variation in tolerance to protect them from chemical contamination. It is necessary to obtain this information before populations decline too precipitously, which would result in oblitera-

tion of potential adaptive variation. An examination of the geographic distribution of historical chemical contamination in relation to distributions of amphibian population declines would also be worthwhile. This information would allow us to correlate declining populations with history of exposure to chemical contamination. Although we know that chemical contaminants can affect amphibian population processes such as juvenile recruitment, whether they do realistically is the ultimate, and as yet, unanswered question.

Chemical hypotheses for amphibian declines are not easy to make because often there is no direct link between contamination and population declines; in fact, many declines have occurred in environments with little or no apparent chemical contamination (e.g., Crump et al. 1992). In fact, it has recently been reported that chytrid fungi may be responsible for many mysterious and sporadic amphibian declines (Carey 1993; Berger et al. 1998; Lips 1998; Kaiser 1999). Although the effects of environmental contaminants on the amphibian immune system are currently unknown (Carey et al. 1999), it is possible that exposure to stressors such as contaminants may depress immune-system function, thus allowing greater susceptibility to fungal infections. Consequently, it is possible that variability in tolerance to environmental stressors may correlate to variation in vulnerability to disease. Furthermore, sublethal levels of chemical contaminants and abiotic factors may interact synergistically to increase the toxicity of compounds (UV-B, Zaga et al. 1998; temperature, Boone & Bridges 1999), or contaminants may break down into more toxic compounds, leading to an overestimation of acceptable field concentrations. Thus, multiple-factor rather than single-factor hypotheses may be necessary to adequately describe the potential effects of chemicals on natural amphibian populations.

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