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Differential swimming performance of two natricine snakes exposed to a cholinesterase-inhibiting pesticide

W.A. Hopkins*, C.T. Winne, S.E. DuRant

University of Georgia, Savannah River Ecology Laboratory, Wildlife Ecotoxicology and Physiological Ecology Program, PO Drawer E, Aiken, SC 29802, USA

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Exposure to a cholinesterase inhibitor reduces swimming velocity in snakes.

Abstract

Environmental contaminants have direct effects on organisms at the molecular, cellular, and tissue levels, but the net results of these sub-organismal effects are only consequential to exposed populations if they alter organism-level traits that ultimately influence fitness (e.g., growth, locomotor performance, reproduction, and survival). Here, we explore the possibility that the swimming performance of neonate black swamp snakes (*Seminatrix pygaea*) and diamondback water snakes (*Nerodia rhombifer*) may be affected by exposure to carbaryl (2.5 and 5.0 mg/L). The highest concentration of carbaryl caused greater reductions in swim velocity in *S. pygaea* than in *N. rhombifer*. Most individuals recovered from the effects of carbaryl on swimming performance within 96 h, but recovery was significantly slower in *S. pygaea* than in *N. rhombifer*. We hypothesize that the sensitivity of *S. pygaea* may arise from its highly permeable integument compared to other natricines. Our findings suggest that performance can serve as an ecologically relevant response to contaminant exposure in reptiles and warrants further study.
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Keywords: Carbaryl; Cholinesterase; Reptiles; Snakes; Swimming performance

1. Introduction

Cholinesterase-inhibiting pesticides (i.e., organophosphates and carbamates) are among the most widely used compounds for pest control in North America (applied at rates of 200 million acre treatments per year in the United States; Hill, 1995, 2003) and pose significant threats to the integrity of both terrestrial and aquatic communities. Despite their rapid degradation and non-bioaccumulative nature, cholinesterase inhibitors can be acutely toxic to a variety of nontarget invertebrates as well as wildlife. As a result of this acute toxicity, periodic mass mortalities have been documented in both terrestrial and aquatic wildlife immediately following pesticide application (Hill, 2003). However, environmental concentrations of cholinesterase inhibitors, particularly in areas offsite of direct application (e.g., runoff into wetlands), are most often not high enough to induce direct mortality, making sublethal responses of paramount importance (Boone and Bridges, 2003).

Traditional toxicological investigations have focused on measuring cholinesterase activity in organisms exposed to organophosphates and carbamates, but such studies provide limited insight into the ultimate ecological ramifications of pesticide exposure. Although measures of cholinesterase activity provide a powerful approach for rapid detection of exposure (i.e., the

* Corresponding author. Tel.: +1 803 952 7427; fax: +1 803 725 3309.
E-mail address: hopkins@srel.edu (W.A. Hopkins).

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biomarker approach) and the mechanistic basis for pesticide toxicity, the net result of this biochemical response is only consequential to exposed populations if it alters organism-level traits that ultimately influence fitness. Evidence suggests that cholinesterase inhibitors can have sublethal effects on a variety of parameters with demonstrated or strong theoretical implications for fitness including growth, behavior, performance, and reproduction (Little et al., 1990; Martin et al., 1991; Bennet and Bennet, 1990; Busby et al., 1990; Sholz et al., 2000; Beauvais et al., 2000; Burkepile et al., 2002). In some cases, reductions in cholinesterase activity correlate well with these important sublethal responses (Beauvais et al., 2000, 2001), but in most instances the relationship between biomarker and organism-level response remains unknown or tenuous (Hart, 1993; Thompson and Walker, 1994; Padilla, 1995; Costa, 1998).

The vast majority of studies on sublethal effects of cholinesterase inhibitors in non-mammalian wildlife have focused on birds and fish, with comparatively little information available on herpetofauna. Fortunately, increased awareness of amphibian population declines has resulted in a recent surge in studies on the effects of pollutants on amphibians, including much recent work on cholinesterase inhibitors. These studies demonstrate that cholinesterase inhibitors can result in a variety of important sublethal effects in amphibian larvae (Bridges, 1997; Bridges and Semlitsch, 2000; Relyea and Mills, 2001; Boone and Semlitsch, 2001, 2002; Boone and Bridges, 2003). In addition, reductions in zooplankton abundance following pesticide application greatly alter amphibian community dynamics and result in indirect toxicity to zooplanktivorous salamander larvae (Boone and James, 2003; Mills and Semlitsch, 2004). In contrast to amphibians, reptiles remain grossly understudied in ecotoxicology and a dearth of toxicological information on organism-level responses to cholinesterase inhibitors exists for these organisms (Campbell and Campbell, 2000, 2001; Hopkins, 2000; Pauli and Money, 2000).

The goal of the current study was to determine whether a cholinesterase inhibitor adversely affects swimming performance in two species of natricine snakes. We used Sevin® (Aventis Group), a commercial formulation containing 22.5% carbaryl (1-napthyl-*N*-methylcarbamate). Carbaryl is a carbamate pesticide of great environmental importance because of its broad spectrum application; of the >21,000 chemical pesticides currently used in the USA (Aspelin, 1997), it is the eighth most commonly used (Larson et al., 1997). Although carbaryl and most other cholinesterase inhibitors are not generally approved for use on wetlands (a notable exception being mosquito-control agents), these compounds enter wetlands by direct overspray, drift, and runoff from high-use areas. Small streams and wetlands are often at highest risk of carbaryl contamination because they are difficult to avoid during aerial application (Hill, 2003). Ironically, small ephemeral wetlands are also some of the most critical and biologically diverse habitats for herpetofauna in North America (Semlitsch and Bodie, 1998). Locomotor performance was selected as an organism-level response to carbaryl because it is a performance measure that may affect prey capture (and therefore acquisition of energy) and predator avoidance (and therefore survival; Christian and Tracy, 1981; Webb, 1986; Huey and Dunham, 1987). Although a wealth of information exists regarding squamate reptile locomotor performance in the ecological literature, particularly for lizards, the use of locomotor performance as an ecologically meaningful endpoint in reptilian ecotoxicology remains practically unexplored (Hopkins, 2000). Therefore, we sought to answer three questions:

(1) Can swimming performance be used as a sublethal response variable in ecotoxicological evaluations of aquatic and semi-aquatic snakes?
(2) Do related species with very different ecological and physiological characteristics respond differently to pesticide exposure?
(3) Do these species exhibit different patterns of recovery from pesticide exposure?

2. Methods

2.1. Species natural history

We selected two species of semi-aquatic snakes (family Colubridae, subfamily Natricinae) with very different ecological and physiological characteristics. While both the diamondback water snake (*Nerodia rhombifer*) and the black swamp snake (*Seminatrix pygaea*) utilize aquatic habitats, *N. rhombifer*, like other *Nerodia*, shuttles frequently between aquatic and terrestrial microhabitats (e.g., adjacent terrestrial habitats and dry perch sites), basks frequently, and ventures into the aquatic environment to forage. In contrast, *S. pygaea* is much more aquatic than other natricines and is not known to bask in terrestrial microhabitats (Gibbons and Dorcas, 2004). The aquatic lifestyle of *S. pygaea* is coupled with physiological adaptations distinct from *Nerodia*. The skin of *S. pygaea* is highly permeable compared to *Nerodia* (specifically *Nerodia fasciata*), resulting in high rates of evaporative water loss (Winne et al., 2001). It is possible that this high skin permeability affords *S. pygaea* with the ability to cutaneously respire, as in other highly aquatic snakes (Heatwole and Seymour, 1978).

2.2. Snake collection and housing

Adult gravid female *N. rhombifer* ($N = 7$; ~80–100 cm SVL) were collected during 28–29 July 2003 from Reelfoot Lake in TN, USA (36°26′39″N, 89°23′09″W). Gravid females were held individually in 284 L aquaria (122 × 46 × 51 cm) in a cold room (~10 °C, 14:10 [light:dark] photoperiod) with sand substrate and water bowls. Substrate thermal gradients (~15 °C–44 °C) were produced within each aquarium using rheostat-controlled heat tape (Flexwatt, Inc.) placed under each aquarium. Snakes were offered previously frozen *Lepomis* sp. once per week (approximately 10–15% of their body mass). After birth (29 August–11 September 2003), neonates from the same litter (approximate litter size = 22 offspring) were housed in 38 L aquaria (25 °C, 14:10 photoperiod) with newspaper bedding and water bowls. Within 3 days of the start of experiments, neonates selected for study were removed from their communal aquaria and housed individually in 737 mL plastic containers (25 °C, 14:10 photoperiod). Mean size of *N. rhombifer* neonates used in the study was SVL = 229.44 ± 1.22 mm, mass = 9.91 ± 0.18 g.

Adult gravid female *S. pygaea* ($N = 16$; ~20–30 cm SVL) were collected at Ellenton Bay (33°13′18″N, 81°44′50″W), an uncontaminated Carolina bay located on the Savannah River Site near Aiken, SC, USA, between 28 May and 30 July 2003. Because *S. pygaea* has husbandry requirements different from those of *N. rhombifer*, gravid females were held individually in plastic 5 L shoeboxes, fitted with paper towels as substrate and a large water dish (737 mL) that allowed the snakes to fully submerge. Water and bedding were changed two to three times per week, and all females were offered ~40–50% of their mass in live salamander larvae (*Ambystoma talpoideum*) every 7–10 days. Following birth (3–17 August 2003, mean litter size = 6.75 offspring), littermates were housed communally (as described for *N. rhombifer*), then prior to the experiment they were housed individually in 236 mL plastic containers with water. All snakes (adult females and neonates) were housed at 25 °C, with a 14:10 photoperiod. Mean size of *S. pygaea* neonates used in the study was SVL = 108.95 ± 0.67 mm, mass = 1.08 ± 0.02 g.

2.3. Experimental design

For our exposure solutions, we used a commercial formulation containing carbaryl (Sevin®). This formulation is one of the most readily available and commonly used forms of carbaryl and has recently become a model formulation for examining effects of cholinesterase inhibitors on amphibian development and life history traits (e.g., Bridges, 1997; Bridges and Semlitsch, 2000; Relyea and Mills, 2001; Boone and Semlitsch, 2001, 2002; Boone and Bridges, 2003; Boone and James, 2003). We chose to work with a common carbaryl formulation because animals are exposed to formulations, not commercial grade compounds, under natural situations (Hill, 2003). All solutions were made within 1 h of use for snake exposures. Solutions were made using weighed quantities of Sevin® dissolved in aerated aged tap water (dissolved oxygen = 9.03 ± 0.10 mg/L; pH = 8.17 ± 0.02; conductivity = 142 ± 1.96 μS/cm). Target carbaryl concentrations of 2.5 and 5.0 mg/L were selected based on previous use of similar concentrations (e.g., Boone and Bridges, 2003; Boone and Semlitsch, 2001). These concentrations represent the upper limits of what is found in surface waters following field application of carbaryl (Norris et al., 1983; Peterson et al., 1994). To verify target concentrations of carbaryl, we sent 5 mL subsamples of water ($N = 3$/trt) to the Mississippi State Chemical Laboratory for analysis. Samples were chilled immediately following preparation and shipped overnight on ice. Samples were analyzed by HPLC with post-column derivatization and fluorescence detection. Actual concentrations in water for the two carbaryl treatments were lower than our nominal concentrations (1.8 and 3.8 mg/L). Control water had no detectable carbaryl (detection limit = 1.0 μg/L).

In the first experiment, 36 snakes of each species were assigned to one of three treatments (0, 2.5, or 5.0 mg carbaryl/L; $N = 12$/species/trt) for 48 h. Snakes were drawn from a pool of six and three litters of *S. pygaea* and *N. rhombifer*, respectively. Allocation of snakes was conducted such that each litter and sex was equally represented among treatments (i.e., stratified random sampling). Each snake was placed in a 1 L flask containing 500 mL of the appropriate test solution. Flasks were randomly arranged on shelves within an environmental chamber (12:12 photoperiod; 25 °C) where they were left undisturbed for 24 h. Because carbaryl breaks down rapidly (half-life = 10 days at neutral pH), test solutions were replaced with new test solution after 24 h, and snakes were returned to the environmental chamber for the last 24 h of the exposure period. At the end of the exposure (total exposure period of 48 h), each snake was removed from its flask (in the random sequence described above) and raced (see below). Following the swim trial, each snake was returned to its respective housing container containing aged tap water (with no carbaryl).

In the second experiment, 24 snakes of each species were assigned to one of two treatments (0 or 5 mg carbaryl/L; $N = 12$/species/trt). Snakes were drawn randomly from a pool of four litters of each species (different litters from those used in experiment 1) such that equal representation of each litter and both sexes occurred between treatments. In this experiment, snakes were raced at five sampling intervals. Immediately prior

to the 48 h exposure period, each snake was raced (see below) to determine its pre-exposure maximum swim velocity. Each snake was then placed in an individual 1 L flask containing 500 mL of the appropriate test solution. Flasks were arranged randomly within an environmental chamber and test solutions changed as described in experiment 1. At the end of the 48 h exposure period, each snake was removed from its flask and raced (see below) to determine their post-exposure swim velocity (equivalent to the post-exposure velocity determined in experiment 1). Following this race, each snake was returned to its individual plastic housing container with 200 mL of aged tap water (with no carbaryl) to recover. Following 6, 24, and 96 h of recovery, snakes were raced again to determine changes in performance over time. All snakes used in both experiments were held for a maximum of 6 weeks following termination of the study before ultimately being released at their natal site.

2.4. Swimming performance

Maximum swim velocity was determined for each snake at each sample interval using a 3-m swim track similar to that previously described in Hopkins et al. (2000, 2003). The track was 8-cm wide (water depth = 3–4 cm), thus encouraging snakes to swim uni-directionally and limiting the influence of side-to-side motion on our swim velocity estimates. Water temperature in the track was maintained at 25 ± 0.5 °C for all swim trials. Two days prior to each experiment, snakes were conditioned to the track by racing each individual three consecutive times.

At initiation of each trial, snakes were rinsed with water (25 °C) to remove any residual carbaryl on their skin before being placed at one end of the track. Control snakes were also rinsed to control for the effects of handling. Snakes were then prodded as frequently as necessary for them to swim the full distance of the track. Each snake was forced to swim three consecutive laps of the track (each lap starting at the same end of the track), resulting in consecutive lap distances of 0–3 m, 3–6 m, and 6–9 m (hereafter referred to as laps 1–3). Swimming occurred over a background marked at 1.0 cm increments and was recorded using a digital video camera (Canon GL1 Mini DV Camcorder). Maximum swim velocity for each lap at each sample interval was later calculated using a frame-by-frame advance on a VCR with accuracy to 0.03 s (Raimondo et al., 1998; Hopkins et al., 2000, 2003). To remove bias from the review process, the identity of snakes was concealed from the video tape reviewer (i.e., tapes were reviewed blindly). The time it took for each individual to swim 30 cm was calculated for each 30 cm segment of the track (after subtracting the initial portion of the track where the snake was placed, a maximum of 261 velocities were possible per lap). The fastest swim velocity (expressed as cm/s; Shine et al., 2003) for each individual during each lap was used for statistical comparisons.

2.5. Statistical analyses

Prior to statistical analysis, data were tested for normality and homoscedasticity using Ryan Joiner and Bartlett's tests, respectively. Although data were normally distributed, variance was not equivalent among treatments or between species ($P < 0.001$) and transformation of data failed to improve this relationship. Therefore, for all comparisons we used a mixed model approach to repeated measures analysis (SAS; PROC MIXED) with a compound symmetry variance matrix structure. Snake SVL was used as a covariate in all analyses.

For experiment 1, we examined the effect of species, pesticide treatment, and swim distance (lap) on swim velocity. Technical difficulties with electronic equipment resulted in one snake (*N. rhombifer*, 5 mg/L treatment) not being recorded, decreasing the sample size in that treatment to 11 individuals. Individual snakes were treated as the random effect and SVL as the covariate in the model. Two- and three-way interaction terms between and among swim distance, treatment, and species were included in the model. Because there was a significant effect of species in the model (see Section 3), we also reran the model for each species separately to examine the effect of pesticide treatment and swim distance on swim velocity.

For experiment 2, our primary objective was to determine if and when snakes recovered from carbaryl exposure. At each of the three swim distances (laps 1–3), we compared pesticide treatments over the five sample intervals (before and after carbaryl exposure, and 6, 24, and 96 h of recovery) for each species using the mixed model approach to repeated measures with SVL as the covariate. We then examined individual parameters in the model using the solutions for fixed effects option in PROC MIXED (i.e., essentially post hoc *t*-tests to determine when snake performance differed over time).

3. Results

3.1. Experiment 1

Species, pesticide exposure, SVL, and swim distance (lap) had significant effects on swim velocity. Overall, *N. rhombifer* swam much faster than *S. pygaea*, even when the effects of body size were accounted for in the statistical model (Fig. 1, Table 1). Moreover, swim velocity was influenced by lap and carbaryl exposure, and was marginally influenced by the three-way

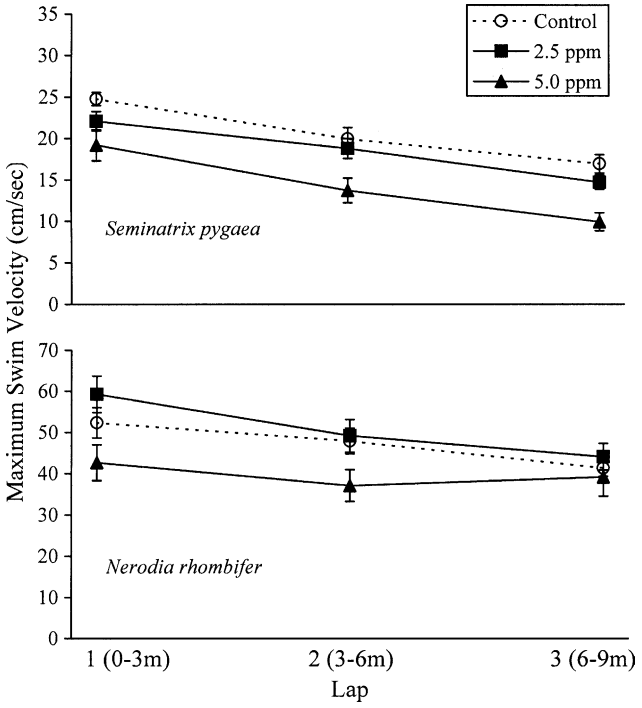

Fig. 1. Comparison of swimming performance of two snake species, *Seminatrix pygaea* and *Nerodia rhombifer*, exposed to three concentrations of carbaryl (0, 2.5, 5.0 mg/L) for 48 h. Maximum swim velocity was determined for each individual over three consecutive swim distances (laps). Note that the *y*-axis differs for the two species. Data are presented as mean ± 1 SE.

interaction among carbaryl, lap, and species ($P = 0.054$). Although the effect of 2.5 mg/L carbaryl was not always discernible from the controls, 5.0 mg/L carbaryl resulted in clear reductions in swim velocity in both species. Compared to controls during the first two laps, *S. pygaea* and *N. rhombifer* exposed to 5.0 mg/L carbaryl exhibited 22–31% and 19–23% reductions in swim velocity (respectively). At the furthest swim distance, however, swim velocity of *N. rhombifer* exposed to 5.0 mg/L carbaryl converged with the controls, but the interaction term was not significant in the statistical model (carbaryl treatment × distance: $P = 0.101$). In contrast, *S. pygaea* exposed to 5.0 mg/L carbaryl diverged furthest from controls (41% reduction in swim velocity) during the third lap.

3.2. Experiment 2

Patterns of response to and recovery from carbaryl differed between the two snake species. Over all three laps, swimming performance of *S. pygaea* was influenced by the interaction between carbaryl and sample interval (Table 2), indicating that pesticide exposure initially reduced swim velocity but velocity re-converged with controls over time (Fig. 2). During the first lap, carbaryl reduced swimming velocity of *S. pygaea* by 35% compared to controls, but recovery of normal swim velocity began to occur within 6 h of exposure and was completed within 96 h (Fig. 2). Post hoc comparisons revealed that treated animals only differed from controls immediately after exposure (Fig. 2). During the second and third laps, carbaryl reduced swimming velocity of *S. pygaea* by 51 and 43% (respectively) compared to controls. Although recovery of swim velocity took longer during these laps compared to the first lap (i.e., post hoc comparisons revealed differences remained between treatments after 24 h of recovery), recovery was clearly completed by 96 h (Fig. 2). In contrast, neither carbaryl nor the interaction between carbaryl and time influenced swimming in *N. rhombifer* (Table 2, Fig. 3). Reductions in swim velocity in *N. rhombifer* due to carbaryl exposure only ranged from 10 to 14% (Fig. 3).

Table 1
Results of repeated measures ANCOVA (mixed model approach) comparing the swimming velocity of two species of snakes exposed to carbaryl over three swim distances (laps)

Effect	Num df	Den df	*F*	*P*
Overall model				
Pesticide treatment	2	64	8.17	<0.001
Lap	2	130	41.15	<0.001
Pesticide treatment × lap	4	130	1.31	0.271
Species	1	64	6.61	0.013
Pesticide treatment × species	2	64	1.85	0.166
Lap × species	2	130	0.69	0.502
Pesticide treatment × lap × species	4	130	2.40	0.054
SVL	1	64	15.54	<0.001
Post hoc comparisons				
Seminatrix pygaea				
Pesticide treatment	2	32	10.10	<0.001
Lap	2	66	54.39	<0.001
Pesticide treatment × lap	4	66	0.46	0.763
SVL	1	32	4.99	0.033
Nerodia rhombifer				
Pesticide treatment	2	31	4.61	0.018
Lap	2	64	14.62	<0.001
Pesticide treatment × lap	4	64	2.03	0.101
SVL	1	31	8.89	0.006

4. Discussion

To our knowledge, this is the first study to examine the effects of a carbamate pesticide in a reptile and the first to explicitly examine locomotor performance as a sublethal measure of contaminant exposure in reptiles. We demonstrated that significant reductions in swimming performance occurred in snakes after aqueous exposure to a commercial formulation containing carbaryl. Moreover, we found significant differences in

Table 2
Results of repeated measures ANCOVA (mixed model approach) comparing the recovery of snakes from carbaryl exposure as a function of sample interval (i.e., time; pre-, post-, +6, +24, +96 h)

Species	Lap	Effect	Num df	Den df	*F*	*P*
Seminatrix pygaea	Lap 1 (0–3 m)	Pesticide treatment	1	21	2.38	0.138
		Time	4	88	1.74	0.149
		Pesticide treatment × time	4	88	2.81	0.030
		SVL	1	21	4.73	0.041
	Lap 2 (3–6 m)	Pesticide treatment	1	21	4.81	0.040
		Time	4	88	5.57	<0.001
		Pesticide treatment × time	4	88	8.13	<0.001
		SVL	1	21	4.19	0.054
	Lap 3 (6–9 m)	Pesticide treatment	1	21	1.77	0.197
		Time	4	88	5.12	<0.001
		Pesticide treatment × time	4	88	5.42	<0.001
		SVL	1	21	6.42	0.019
Nerodia rhombifer	Lap 1 (0–3 m)	Pesticide treatment	1	21	3.33	0.082
		Time	4	88	3.18	0.017
		Pesticide treatment × time	4	88	0.88	0.477
		SVL	1	21	1.22	0.282
	Lap 2 (3–6 m)	Pesticide treatment	1	21	0.49	0.490
		Time	4	88	0.91	0.459
		Pesticide treatment × time	4	88	0.90	0.466
		SVL	1	21	0.41	0.527
	Lap 3 (6–9 m)	Pesticide treatment	1	21	0.80	0.380
		Time	4	88	7.23	<0.001
		Pesticide treatment × time	4	88	0.71	0.584
		SVL	1	21	2.29	0.145

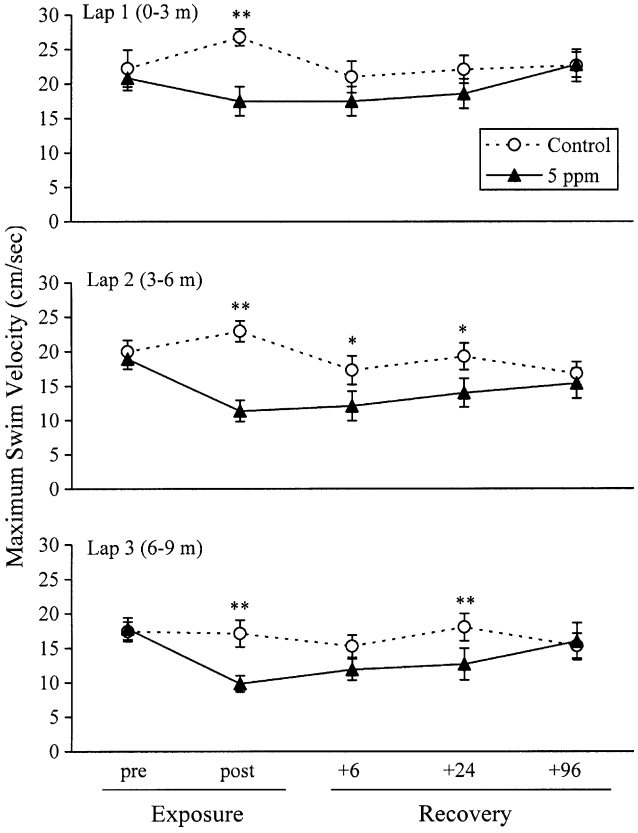

Fig. 2. Swimming performance of *Seminatrix pygaea* exposed to two concentrations of carbaryl (0 or 5.0 mg/L) for 48 h. Maximum swim velocity was determined for each snake prior to exposure, immediately after exposure, and at three time intervals (6, 24, and 96 h) during recovery. At each sampling period, swim velocity was determined for each individual over three consecutive swim distances (laps). Data are presented as mean ± 1 SE. * and ** denote a significant difference ($P < 0.05$ and $P < 0.01$, respectively) between control and treated snakes (from post hoc *t*-tests).

sensitivity between species, suggesting that manifestation of this sublethal response may vary among species with different biological traits.

In light of carbaryl's biochemical mode of toxicity, a reduction in swimming performance is a logical translation upwards from the biochemical to the organism level. Like other cholinesterase inhibitors, carbaryl binds to cholinesterase enzymes (e.g., acetylcholinesterase) and forms a stable complex that breaks down slowly (Landis and Yu, 1995; Thompson and Walker, 1994). This binding prevents the enzyme from breaking down its target neurotransmitter (e.g., acetylcholine), resulting in hyperexcitation of the receiving nerve or muscle fiber. Thus, disruption of normal signals across neuromuscular junctions likely results in the observed effects on locomotor performance. It is important to note that carbaryl is among the least toxic carbamate pesticides to wildlife (Hill, 2003), so it is certainly possible that other widely used carbamates (e.g., aldicarb, carbofuran) or organophosphates would produce similar, and perhaps even more pronounced, responses in snakes.

The few other studies that exist on the effects of cholinesterase-inhibiting pesticides on reptiles are almost exclusively focused on the biochemical response of exposed individuals, and rarely consider the organism-level consequences of these enzymatic disruptions. Studies by Hall and Clark (1982), Sanchez et al. (1997), and Sanchez-Hernandez (2003) have laid the crucial foundation for using inhibition of acetylcholinesterase and butyrylcholinesterase as biomarkers in reptiles, but the ramifications of their findings for higher levels of biological organization remain untested. However, two recent studies evaluated the effects of pesticides on prey capture in lizards, an endpoint that incorporates visual acuity, neurosensory and motivational (i.e., hunger) components, as well as muscle coordination (Peveling and Demba, 2003; Bain et al., 2004). Although Bain et al. (2004) were unable to interpret the highly variable feeding performance of lizards exposed to fenitrothion (an organophosphate),

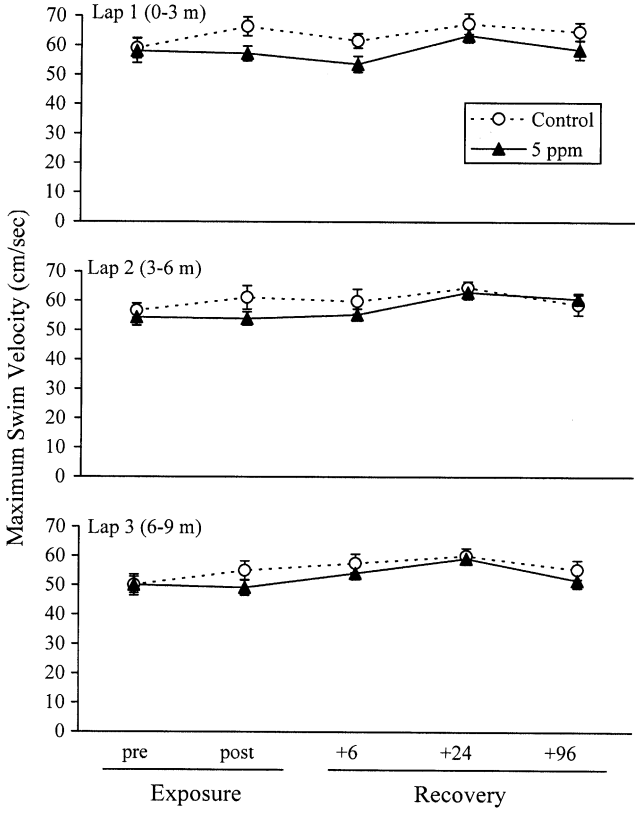

Fig. 3. Swimming performance of *Nerodia rhombifer* exposed to two concentrations of carbaryl (0 or 5.0 mg/L) for 48 h. Maximum swim velocity was determined for each snake prior to exposure, immediately after exposure, and at three time intervals (6, 24, and 96 h) during recovery. At each sampling period, swim velocity was determined for each individual over three consecutive swim distances (laps). Data are presented as mean ± 1 SE.

Peveling and Demba (2003) clearly demonstrated reduced feeding activity in lizards exposed to fipronil (a phenylpyrazole pesticide). In light of the fact that reptiles are declining around the globe at alarming rates (Gibbons et al., 2000), our findings and the results of these recent studies suggest that future consideration should be given to integrated, whole animal responses to pesticides with particular emphasis on those related to reproduction and survival.

Perhaps the most interesting result of our study was the clear difference observed between species. Overall, *S. pygaea* was much more sensitive to the effects of carbaryl on swimming performance than *N. rhombifer* (Figs. 1–4). Direct comparison of data from experiment 1 to the post-exposure data collected in experiment 2 reveals that this observation was consistent between experiments, but the difference between species was even more profound in the second experiment (Fig. 4). The accentuation of the difference between species was caused by higher sensitivity of *S. pygaea* used in experiment 2 compared to experiment 1, while the opposite was true for *N. rhombifer* (Fig. 4). Although this study was not designed to examine genetic variation in responsiveness to carbaryl (i.e., only two to four individuals per litter were assigned to each treatment), visual inspection of the data suggests that substantial inter-litter variation in pesticide sensitivity in both species contributes to the observed differences between experiments. Such inter-litter variation in responsiveness is not unprecedented; Bridges and Semlitsch (2000) demonstrated significant inter-clutch variation in tadpole swimming performance following carbaryl exposure.

The difference in responsiveness between species is likely the result of differences in physiological sensitivity, dose received, or a combination of these factors. Physiological sensitivity to cholinesterase inhibitors can vary widely both among, and within, species (e.g., Bridges and Semlitsch, 2000; Van Dolah et al., 1997). However, if physiological sensitivity is similar between species, responses of species could still differ if other factors influence the dose of contaminant reaching the

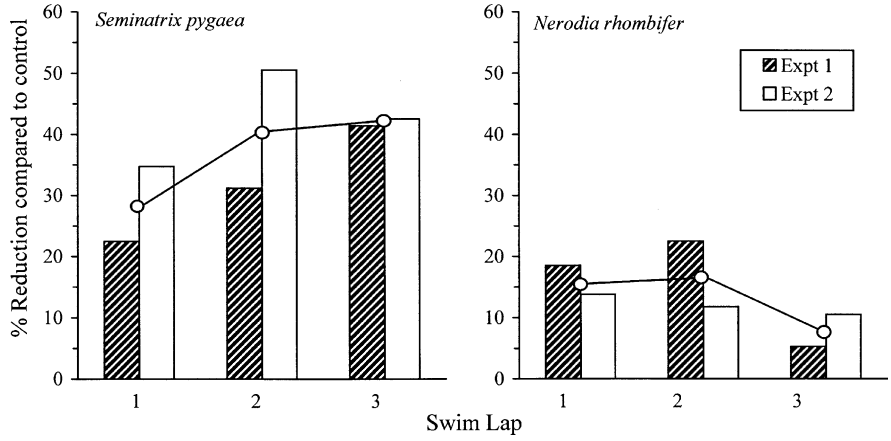

Fig. 4. Swimming performance of two snake species, *Seminatrix pygaea* and *Nerodia rhombifer*, exposed to 5 mg/L carbaryl expressed as percent reduction compared to controls. Paired bars represent comparison of data collected immediately after 48 h of exposure to appropriate test solutions in experiments 1 and 2. Points overlaid on graph represent the overall mean of paired bars (i.e., data from both experiments pooled).

site of toxic action (i.e., cholinesterases). The amount of carbaryl reaching cholinesterases in this study was likely primarily governed by the amount of compound that crossed the integument. In turn, integument transfer would likely be influenced by the inherent permeability of the skin to water and the surface area-to-volume ratio (SA/V) of the organism. We hypothesize that the small body size (and therefore high SA/V) and highly permeable integument of *S. pygaea* (Winne et al., 2001), alone or in combination, contribute to the sensitivity of this organism. Interestingly, the aquatic lifestyle of *S. pygaea* would also result in greater exposure to dissolved pesticides in nature compared to other more semi-aquatic natricines that spend less time in water.

Although performance in carbaryl-treated snakes was reduced by as much as 51% compared to controls, both species regained their swimming performance within 4 days of exposure. This finding is in general agreement with other studies of behavior and performance in wildlife that show that recovery often occurs within days of acute exposure to carbamates and organophosphates (e.g., Dell'Omo and Shore, 1996; Hill, 1995). In contrast, Bridges (1997) found that amphibian larvae exposed to carbaryl had not regained their normal swimming capacity after a 48 h recovery period. However, amphibians in that study had regained normal spontaneous activity levels within 48 h of exposure, suggesting that recovery was underway (Bridges, 1997). These findings are important because they suggest that the timing of pesticide application relative to the biology of snakes and other wildlife is extremely important. Pesticide applications that occur during critical windows of activity (e.g., the breeding season, parturition, or upon emergence from dormancy) might have a greater effect than applications occurring during less critical portions of the organism's annual cycle. Indeed, evidence suggests that cholinesterase inhibitors applied during the reproductive season of birds and the migratory season of fish could have pronounced effects on reproduction and homing behavior, respectively (Busby et al., 1990; Sholz et al., 2000).

Our findings raise a number of questions that warrant further study. For example, additional evaluations of inter- and intra-specific differences in swimming performance of snakes exposed to cholinesterase inhibitors will help elucidate the factors involved in differential sensitivity between species. Such studies will obviously be extremely important from a conservation perspective, as identifying sensitive populations and/or species can form the foundation for sound management plans. Moreover, because cholinesterase-inhibiting pesticides are often applied repeatedly during each growing season (Hill, 2003), longer term (e.g., 1–2 weeks), repeated exposures may have greater ecological relevance than a single brief exposure (Boone and Bridges, 2003) and should be examined. The exposure concentrations utilized in our study also represent high environmental concentrations, so studies examining repeated exposure to lower concentrations would be of particular value. Finally, the implications of reduced performance for processes such as predator avoidance and prey capture will be crucial in further assessing the ecological importance of our findings.

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