Sublethal effects of an acetylcholinesterase-inhibiting pesticide on fitness-related traits in the western fence lizard (*Sceloporus occidentalis*)

Sarah Elizabeth DuRant

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Committee Chair: William A. Hopkins
Committee Member: Eric M. Hallerman
Committee Member: Thomas A. Jenssen

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Abstract

Pesticides are commonly used around the world for a multitude of different purposes and on diverse habitats, including agricultural fields, wetlands, and personal lawns and gardens. Currently, acetylcholinesterase (AChE)-inhibiting pesticides are among the most prevalently used chemical pesticides in the United States. A wealth of information exists on sub-cellular responses of organisms, primarily birds, mammals, and fish, exposed to these compounds. However, the effects of AChE-inhibiting pesticides at the whole-organism level, most importantly effects relevant to an individual’s fitness, have received less attention. My Master’s research focused on describing the effects of carbaryl, an AChE-inhibiting pesticide, on several fitness-related traits in the western fence lizard (*Sceloporus occidentalis*). Reptiles are the least studied vertebrate taxon in ecotoxicological studies even though contaminants are suspected in contributing to recent population declines. Using multiple dose concentrations within the range expected to occur in nature (based on EPA application rates and published pesticide residues on insects), I quantified the effects of carbaryl on sprint performance energy acquisition, and energy allocation, traits which could have important implications for the animal’s ability to avoid predators, capture prey, and grow and reproduce. I found that at the highest dose concentration, lizards experienced a decrease in arboreal and terrestrial locomotor performance, a decrease in energy acquisition, and alterations in energy allocation. My findings suggest that acute exposure to high concentrations of carbaryl can have important sublethal consequences on fitness-related traits in *S. occidentalis*. Future studies should examine the consequences of multiple-pulse exposures to AChE-inhibiting pesticides on reptiles.
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Literature Review

Introduction

Since the industrial revolution, humans in developed nations have drastically changed their way of living. Houses use electricity, transportation is achieved by the use of combustion engines, and agricultural practices are conducted on much larger scales. Unfortunately, many of these changes have greatly impacted the environment. The progress of society has not occurred without costs, and often fish and wildlife bear the greatest burden.

One agricultural practice that carries significant ecological costs is the use of pesticides. Pesticides are used throughout the world and are often applied over large land areas via aerial spraying. Because of their widespread use and methods of application, an abundance of non-target wildlife populations are exposed to these contaminants. Adverse responses of wildlife to pesticides have led to the banning of some compounds, like DDT. However, the disuse of one type of pesticide often requires an increase in the use of alternatives because of our dependence on pesticides for large-scale agricultural production. Although newer pesticides tend to be less persistent in the environment, they do not come without risks. While some risks are due to the acute lethal toxicity of the pesticides to the affected animal, more often the risks include sublethal changes in performance or physiological processes that can ultimately impact the animal’s fitness. Although it is reasonable to assume that I will continue to use chemicals to control insects and other agricultural pests, it is necessary that I simultaneously minimize the impact of pesticides on non-target wildlife. Therefore, it is important that I understand both the lethal and sublethal effects of pesticides on non-target wildlife in order to make informed decisions about which compounds are least hazardous, which species may be at the greatest risk, and alternative application practices.

Acetylcholinesterase-inhibiting pesticides

Some of the most commonly-used pesticides in the United States are those that inhibit acetylcholinesterase (Donaldson 2004). Their use became popular after the banning of DDT (O'Brien 1967) and they are used on a variety of different habitats including wetlands, agricultural fields, and home lawns and gardens (Hill 1995).
Acetylcholinesterase-inhibiting compounds are neurotoxicants that deactivate acetylcholinesterase (AChE), the enzyme responsible for breaking down acetylcholine (Ach) which is a neurotransmitter found at neuromuscular junctions (Fukuto 1990). Inhibition of AChE leads to the build-up of Ach, which can cause hyper-stimulation of muscle tissue leading to paralysis (Koelle 1994). At high doses, AChE-inhibitors can lead to death resulting from respiratory failure (Norris et al. 1983). Most AChE-inhibiting pesticides are divided into two different categories, organophosphates and carbamates; they differ in the reversibility of their effects (Fukuto 1990). Carbamates temporarily inhibit AChE, and the recovery of the enzyme can occur within 30 minutes to several hours or days (O'Brien 1976; Fukuto 1990). In contrast, organophosphates tend to irreversibly bind AChE and recovery occurs only with new synthesis of AChE (O'Brien 1967).

Impacts of AChE-inhibiting pesticides on wildlife

Exposure of non-target wildlife is a common and unfortunate consequence of the use of AChE-inhibiting pesticides (reviewed by Grue et al. 1991). Both sub-lethal and lethal effects of these pesticides have been noted in a variety of bird, fish, and mammal species.

In birds, exposure to AChE-inhibiting pesticides has resulted in decreased nest success and egg laying, not only in laboratory experiments, but also in birds inhabiting conventionally treated agricultural areas (Busby et al. 1990; Bennett et al. 1991; Fluetsch and Sparling 1994; Grue et al. 1997). AChE-inhibiting pesticides also have been linked to changes in normal reproductive behavior, such as decreased song production in passerines (Patnode and White 1991). Increased predation upon individuals exposed to these pesticides was observed in northern bobwhite (Buerger et al. 1991).

In mammals, reduced reproductive productivity was noted in four small mammal species held in experimental small mammal communities exposed to diazinon, an organophosphate (Sheffield and Lochmiller 2001). Exposure to diazinon also appeared to alter normal competitive relationships between two of the species involved in the study. Decreased arousal and motor activity was noted in rats exposed to chlorpyrifos
(Nostrandt et al. 1997). Similar effects also were noted in the pine vole, wood mouse, and common shrew (Durda et al. 1989; Dell’Omo and Shore 1996; Dell’Omo et al. 1997)

Similar findings have been noted in fishes that have been exposed to AChE-inhibiting pesticides. AChE-inhibiting pesticides were found to impair homing behavior and antipredator alarm responses in Chinook salmon (Scholz et al. 2000) and decreased spontaneous swimming rates and feeding rates in coho salmon (Sandahl et al. 2005). Decreased swimming rates and distances also were noted in rainbow trout (Beauvais et al. 2000), and altered feeding behavior was noted in bream (Pavlov et al. 1992).

**Reptiles and ecotoxicology**

During the last several decades, scientists have become acutely aware of declining reptile populations around the world (Gibbons et al. 2000). Documenting the decline in reptiles, however, has been problematic because of a lack of long-term data, the reclusive nature of some reptiles, low densities of natural population, and periods of inactivity (Parker and Plummer 1987; Gibbons et al. 2000). According to the Partners of Amphibian and Reptile Conservation (PARC), six factors are suspected as contributing to these declines: habitat loss and degradation, introduced invasive species, global climate change, disease and parasitism, unsustainable harvest, and environmental pollutants.

Although contaminants are suspected in contributing to reptile declines, reptiles remain the least studied vertebrate taxon in ecotoxicological studies (Hopkins 2000). Most of the knowledge concerning contaminants and reptiles comes from studies involving alligators and turtles, while lizards and snakes have received less attention. However, a recent review (Campbell and Campbell 2002) of contaminant research on lizards and snakes revealed gaps in existing knowledge, and outlined opportunities for future research, including studies relating exposure rates and concentrations to effects.

Furthermore, most studies involving reptiles have focused on sub-cellular responses to contaminants and tissue residues of toxicants (Hopkins 2000, 2006). Although these studies are of great value in understanding the mechanistic basis for toxicity, they do not lend insight into the impact contaminants have on traits that are significant to the fitness of an individual (Adams et al. 1989; Hopkins 2005; Schloz and Hopkins 2006). Alterations in the reproductive output and survival of individuals can
ultimately lead to changes in the dynamics of natural populations. Therefore, conservation initiatives for reptiles could benefit from studies examining organism-level effects of contaminants with known consequences for survival and reproduction.

**Carbaryl**

The pesticide I used in my study was carbaryl (1-naphthyl methylcarbamate). Carbaryl belongs to the class of AChE-inhibiting pesticides called carbamates. Carbamates deactivate AChE through carbamylation, in which a carbamyl group binds reversibly to the active site of the enzyme (Fukuto 1990). Carbamate-inhibited AChE is not stable, however, and the carbamyl group is quickly hydrolyzed, resulting in recovery of the enzyme generally within 30 minutes to several days (O'Brien 1976; Fukuto 1990).

I chose carbaryl for my study because it is one of the most widely used non-commercial insecticides. According to the US EPA’s Pesticide Industry Sales and Usage 2000-2001 Market Report, carbaryl was ranked as the sixth most-commonly used conventional pesticide in the home and garden market. The 22.5 % formulation I chose (Sevin®; Garden Tech, Lexington, KY, USA) is available for non-commercial use. Carbaryl is used on a variety of agricultural crops, including but not limited to oranges, apples, alfalfa, tree nuts, and turfgrass (EPA 2004). Rates of carbaryl application range from 1.0—22.42 kg of active ingredient/ha, but it is most commonly applied at a rate of 8 kg of active ingredient/ha (EPA 2004). Carbaryl can be applied 1—8 times a season, with the time between applications ranging from 7—30 days (EPA 2004). Carbaryl is a relatively short-lived contaminant in the environment, with a half-life in soil and water between 2—9 days (Marutani and Endirveersingham 2003). The short half-life of this contaminant makes studies of acute exposure to carbaryl ecologically relevant.

Carbaryl is among the least toxic carbamates to wildlife, but studies have shown that exposure to carbaryl can decrease activity in tadpoles (Bridges 1997) and reduce swimming performance in water snakes (Hopkins et al. 2005a; Hopkins and Winne 2006). Carbaryl also has been shown to have significant impacts on simulated amphibian communities. Carbaryl application resulted in reductions of zooplankton, reduced survival to metamorphosis in toads and tree frogs, high mortality in salamander larvae, increased competition and predation in leopard frog communities, and reduced species
richness in experimental ponds (Boone and Semlitsch 2001; Boone and James 2003; Boone et al. 2004; Mills and Semlitsch 2004; Relyea 2005).

Sceloporus occidentalis

I used the western fence lizard, Sceloporus occidentalis to examine the effects of carbaryl on whole-organism responses because Sceloporus lizards are common to North America and inhabit regions of the country that are exposed to high rates of pesticide use (e.g., San Joaquin Valley, CA, USA). Sceloporus belong to the family Phrynosomatidae. This family accounts for more than 30% of all lizard species in the United States. Some Sceloporus serve as good study organisms because their entire life cycle is manageable in the laboratory, and a great deal is known about their ecology, physiology, performance and life history (Sinervo and Adolph 1989; Vanberkum et al. 1989; Garland et al. 1990; Huey et al. 1990; Sinervo 1990; Sinervo 1990; Sinervo and Huey 1990; Bennett et al. 1991; Sinervo et al. 1991; Sinervo and Losos 1991; Klukowski and Nelson 1998; Angilletta et al. 2002; Talent et al. 2002). The species used in this study, S. occidentalis, ranges from Mexico to Canada and between the California coast and western Utah, USA. My laboratory stock originated from a population in the grasslands of the San Joaquin Valley, CA, USA. Most females from this population reach sexual maturity within one year under ad libitum feeding conditions in the laboratory, and lay 3—6 clutches of 8—15 eggs per year. This population of western fence lizards does especially well under laboratory conditions and has been identified as a good candidate for use as a laboratory reptile model in ecotoxicology studies (Talent et al. 2002; Hopkins et al. 2005b). Sceloporus occidentalis is also primarily insectivorous, making feeding on contaminated insects an important mode of exposure to pesticides for this species.

Assessment of the effects of carbaryl on fitness-related traits

For my Master’s research I examined the effects of carbaryl on several different traits that influence the probability of survival and reproduction in individuals. To do this, I conducted four separate experiments. In the first two experiments, I examined the effects of carbaryl on sprint performance, a trait which can have important implications on the ability of an individual to avoid predators (i.e., survive) and to capture prey (i.e.,
acquire energy). In the second two experiments, I examined the effects of carbaryl on energy expenditure and acquisition, traits which are important for processes such as growth and reproduction.
Chapter 1
Impaired terrestrial and arboreal locomotor performance in the western fence lizard (Sceloporus occidentalis) after exposure to an AChE-inhibiting pesticide.
Abstract

I examined the effects of a commonly used AChE-inhibiting pesticide on terrestrial and arboreal sprint performance, important traits for predator avoidance and prey capture, in the western fence lizard (*Sceloporus occidentalis*). Lizards were exposed to carbaryl (2.5, 25, and 250 µg/g) and were raced before and 4, 24, and 96 hrs after dosing. Exposure to the highest dose resulted in a decrease in sprint speed in both the arboreal and terrestrial settings, but reductions in sprint speed were twice as great in the arboreal setting. Lizards in the highest dose group were also 4.5X and 7.5X more likely than controls to refuse to traverse or fall off the arboreal substrate, respectively. My findings suggest that acute exposure to high concentrations of carbaryl can have important sublethal consequences on fitness-related traits in reptiles and that arboreal locomotor performance is a more sensitive indicator of AChE-inhibiting pesticide poisoning than terrestrial locomotor performance.

Introduction

Although acetylcholinesterase (AChE)-inhibiting pesticides are among the most commonly used pesticides in the United States (Kiely 2004), their impact on wildlife, especially reptiles, is not thoroughly understood. The use of AChE-inhibiting pesticides became popular because they are relatively short-lived in the environment and do not bioaccumulate (Hill 1995). Unfortunately, the use of these pesticides does not come without costs. Numerous studies have shown that acute exposure to AChE-inhibiting pesticides can have adverse effects on non-target wildlife (Busby et al. 1990; Buerger et al. 1991; Hart 1993; Fryday et al. 1994; Bridges 1997; Brunet et al. 1997; Grue et al. 1997; Beauvais et al. 2000; Scholz et al. 2000; Relyea 2005; Sandahl et al. 2005). While the majority of studies examining the effects of AChE-inhibiting pesticides have focused on birds and fish, relatively few studies have examined herpetofauna (Hopkins 2000). Recently, the effects of AChE-inhibiting pesticides on amphibians has received attention (Boone and Bridges 2003; Rohr et al. 2003; Boone et al. 2004; Metts et al. 2005; Relyea, 2005), but few studies have focused on effects in reptiles (Hopkins 2000; Campbell and Campbell 2002; but see Bain et al. 2004; Hopkins et al. 2005b; Holem et al. 2006; Hopkins and Winne 2006). In fact, reptiles, particularly lizards and snakes, remain the
least studied vertebrate taxa in ecotoxicological studies even though contaminants have been implicated as one factor contributing to reptile population declines (Gibbons et al. 2000). Of particular importance is understanding the effect of AChE-inhibiting pesticides on traits relevant to the individual’s fitness (Hopkins 2000, 2005a; Campbell and Campbell 2002).

In lizards and snakes, one important fitness-related trait that could be affected by exposure to AChE-inhibiting pesticides is locomotor performance. Sprint speed is a commonly measured endpoint in ecological and evolutionary studies of lizards due to its apparent importance to survival and fitness (Garland et al. 1990; Garland and Losos 1994; Warner and Andrews 2002; Miles 2004; Husak 2006; Peterson and Husak 2006) and because it is a highly repeatable measure in a laboratory setting (Huey and Dunham 1987; Vanberkum et al. 1989). Several studies involving reptiles and amphibians have found that exposure to AChE-inhibiting pesticides can greatly impact an individual’s ability to perform (Bridges 1997; Hopkins et al. 2005b; Hopkins and Winne 2006). While exposure to pesticides may not directly result in death, reductions in performance could ultimately be of equal importance to the individual’s fitness by altering an individual’s ability to avoid predators, capture prey, and/or defend territories.

In my study I sought to examine the effects of carbaryl, an AChE-inhibiting pesticide, on sprint performance in both terrestrial and arboreal settings in *Sceloporus occidentalis* (western fence lizard). Two studies on the effects of carbaryl on swimming speed in four species of Natricine snakes documented 19 – 31 % decreases in swimming performance during the first 24 hrs after exposure, but animals recovered within 96 hrs after exposure (Hopkins et al. 2005b; Hopkins and Winne 2006). Exposure to carbaryl also resulted in a decrease in swimming speed and distance in leopard frog tadpoles (*Rana blairi*) (Bridges 1997). Based on the results of these previous studies, I hypothesized that exposure to carbaryl would result in a dose-dependent decrease in sprint speed in lizards. I also predicted that sprint speed in an arboreal setting would prove to be a more sensitive endpoint to the effects of carbaryl than terrestrial performance because arboreal locomotor performance requires greater coordination and balance than running on a flat surface (Losos and Sinervo 1989; Irschick and Losos 1999; Mattingly and Jayne 2005).
**Materials and Methods**

**Carbaryl and Dose Administration**

The pesticide chosen for my study was carbaryl (1-naphthyl methylcarbamate). Carbaryl, is a carbamate which deactivates acetylcholinesterase through carbamylation, during which a carbamyl group binds reversibly to the active site of the enzyme (Fukuto 1990). Carbamate-inhibited AChE is not stable, however, and the carbamyl group is hydrolyzed within minutes to days, resulting in recovery of the enzyme (O'Brien 1976; Fukuto 1990).

Carbaryl is one of the most widely used non-commercial insecticides. According to the US EPA’s Pesticide Industry Sales and Usage 2000 - 2001 Market Report (Kiely 2004), carbaryl was ranked as the sixth most commonly used conventional pesticide in the home and garden market. The 22.5 % formulation I chose, Sevin ©, is available for non-commercial use. Carbaryl is used on a variety of agricultural crops such as oranges, apples, alfalfa, tree nuts, and turfgrass (EPA 2004). Carbaryl can be applied between 1 - 8 times a season with the time between applications ranging from 7 - 30 days (EPA 2004). Carbaryl is a relatively short-lived contaminant in the environment with a half-life in soil ranging between 8 - 18 days (Nkedi-Kizza and Brown 1998). The short half-life of carbaryl makes studies of acute exposure ecologically relevant.

Although contaminated insects are an important route of exposure to pesticides for insectivorous vertebrates inhabiting areas that receive pesticide application, very few data exist on invertebrate pesticide residues. Therefore, it is difficult to predict actual concentrations lizards are likely to encounter in the wild. In the only study to directly quantify carbaryl residues of terrestrial invertebrates, Fair et al. (1995) showed that grasshoppers had mean residues of 17 µg/g two days following rangeland application of 0.5 kg active ingredient/ha. Using this insect residue data and carbaryl application rates, which can vary from 1.12 to 22.42 kg active ingredient/ha (EPA 2004) I estimated that a 10 g lizard consuming 1 g of prey could ingest dose concentrations ranging between 3.9 – 78.5 µg/g 2 days following carbaryl application. Factoring the short half-life of carbaryl into my estimates, I selected three doses that fully encompass the range of concentrations that lizards could encounter in the environment.
Carbaryl was administered to lizards via oral gavage using an Eppendorf micropipette (2 – 20 µl). The 2.5 and 25 µg/g solutions were made within one hour of administration by diluting the 22.5% Sevin © formulation with water and then vortexing the solution for one minute. Lizards in the 250 µg/g treatment group received an undiluted dose of the 22.5% Sevin © formulation, and lizards in the oral gavage treatment group received a comparable volume of water. A control group (i.e., not receiving gavage) was not used in these experiments because prior research demonstrated that lizards gavaged with water did not differ in sprint speed from unmanipulated control animals (Holem et al. 2006).

Fence Lizard Natural History and Husbandry

_Sceloporus_ lizards belong to the family Phrynosomatidae which accounts for more than 30% of all lizards in the United States. The species used in my study, _S. occidentalis_, ranges from Mexico to Canada between the California coast and western Utah, USA. The original parental stock of western fence lizards used in my study originated from a population in the grasslands of the San Joaquin Valley, CA, USA. Most females reach sexual maturity within one year under *ad libitum* feeding conditions in the laboratory, and lay 3 - 6 clutches of 8 - 15 eggs per year. This population of western fence lizards does especially well under laboratory conditions and has been identified as a good candidate for use as a laboratory reptile model in ecotoxicology studies (Talent et al. 2002; Hopkins et al. 2005c).

Adult western fence lizards, representing the F2 generation, were shipped to Savannah River Ecology Laboratory (SREL) from a breeding colony at Oklahoma State University. Lizards husbandry protocol was identical to that of Hopkins et al. (2005c) with the following exceptions: a 10:14 (light: dark) photoperiod, a daytime temperature gradient within each lizard’s cage of ~28—40°C, and a diet consisting of 4 crickets (~1.5 cm each) a day.

Experiment I: Terrestrial Locomotor Performance

In my first experiment I sought to determine the effects of carbaryl exposure on terrestrial sprint velocity. Lizards were fasted 48 hrs before the start of the experiment
after which time they were assigned to one of four treatment groups: gavage control, 2.5 µg/g, 25 µg/g, or 250 µg/g carbaryl (N = 10 males and 10 females / treatment). Sprint velocity was measured at four time intervals, before dosing, and 4 hrs, 24 hrs, and 96 hrs after dosing using a 2.3 m linear sprint track lined with pairs of photocells projecting infrared beams at 10 cm intervals interfaced with a laptop computer (Columbus Instruments, Columbus, Ohio, USA). Methods are similar to Holem et.al (2006). All lizards were conditioned to the sprint track before the start of the experiment by racing the lizard down the track 2 times 24 hrs prior to the start of the experiment. At each time interval lizards were raced successively for a total of 3 laps. Body mass of lizards used in the terrestrial sprint trials ranged between 11.0 - 22.1 g and dose volumes ranged between 11.3 - 22.6 µL. Because temperature influences sprint speed, lizards were maintained at their optimal body temperature (34 ± 1°C) during sprint trials (Bennett 1980; Crowley 1985; Huey and Bennett 1987) and their temperature was recorded prior to each lap using a Scultheis ® cloacal thermometer. Lizards were offered five crickets after the 24 hr sprint time interval and any remaining crickets were removed 24 hrs later.

For each lap at each time interval I calculated an individual’s maximum terrestrial velocity (MTV) over each 0.2 m interval. Lizard performance was estimated using two methods. In the first method, I used the greatest MTV of the three laps, which is similar to techniques commonly used in the literature (Huey and Dunham 1987; Van Berkum et al. 1989; Sinervo and Losos 1991; Irschick and Losos 1999; Holem et al. 2006). However, I observed that while some lizards performed very well on the first lap, performance declined on the second and third laps. In the second method, I monitored MTV of each lap per time interval. This allowed us to monitor how the performance of a lizard changed over the entire time interval. Similar techniques used with snakes revealed that exposure to carbaryl resulted in a decline in swimming performance with each subsequent lap (Hopkins et al. 2005b).

**Experiment II: Arboreal Locomotor Performance**

To better understand the effects of carbaryl on locomotion in lizards, I also examined how lizards performed in an arboreal setting (artificial branch). Several studies have indicated that sprint performance differs depending on the substrate (Losos and
Sinervo 1989; Sinervo and Losos 1991; Mattingly and Jayne 2004). Testing lizards in an arboreal setting examined the potential effects of carbaryl on finer motor skills (balance, ability to grasp) that might not be apparent in a terrestrial setting. To measure arboreal performance, I raced lizards for two consecutive laps at the four aforementioned time intervals on a 1.2 m wood dowel rod; 2.54 cm in diameter marked at 10 cm intervals and covered with 1 cm² fiberglass mesh screening. The dowel rod was horizontally suspended ~ 2 m above the ground and a hidebox was positioned at the finish to encourage lizards to run across the rod. Similar to the terrestrial performance experiment, lizards were fasted 48 hrs prior to treatment administration; pre-treatment trials occurred immediately prior to dose administration, and lizards were raced at 34 ºC. Lizards used in this experiment were also offered five crickets after completion of the 24 hr time interval sprint and remaining crickets were removed 24 hrs later.

I calculated lizard’s maximum arboreal velocity (MAV) per lap per time interval over a 0.2 m interval using a frame by frame advance on a VCR (30 frames per second) (Hopkins et al. 2005b). Lizard arboreal performance was estimated similarly to terrestrial performance. Each treatment group consisted of 10 lizards per sex, except for the low dose group that contained 10 females and 11 males. Body mass of adult lizards in the arboreal sprint trials ranged from 10.5—19.2 g and dose volumes were ranged from 10.8—19.7 µL.

**Statistical Analyses**

Maximum terrestrial velocity and MAV achieved at each time interval were analyzed separately using a mixed model approach (SAS PROC MIXED) with snout-vent length (SVL) as the covariate. The initial models included all interaction terms but insignificant interactions were dropped in subsequent iterations of the model. Initial models indicated there was no difference in sprint speed between males and females, and therefore sexes were combined for statistical analyses. Maximum terrestrial velocity and MAV achieved for each individual lap at each time point were analyzed separately in an identical manner. Some individuals in the arboreal performance experiment refused to run for one or both laps during a sprint trial; these individuals were not included in statistical analyses.
I also examined the number of lizards that refused to traverse or fell off of the arboreal substrate at least once during the arboreal experiment. These calculations were then compared among treatment groups using a Fisher’s Exact Test.

Results

The dose concentrations administered to lizards in the terrestrial and arboreal experiments did not result in any mortality. However, 58% (23/40) of lizards in the highest dose group exhibited clinical signs of exposure to carbaryl (e.g., body/limb tremors, twitching). Onset of tremors began as early as 4 hrs after exposure and persisted up to 48 hrs after exposure.

Maximum Terrestrial Velocity achieved at each time interval of lizards was significantly affected by treatment, but this was also dependent on time (treatment X time: $F_{9,228} = 2.07, p < 0.0331$). The treatment and time effects were most likely caused by an 11 - 23% increase in sprint speed at the lower concentrations at 4 and 24 hrs after exposure and a 9 - 10% decrease in sprint speed at the highest dose concentration at 4 and 24 hrs after exposure (data not shown). Similarly, when I examined MTV of each lap separately I found there was a significant treatment X time interaction for each lap and this effect became more pronounced with each subsequent lap (lap 1: $F_{9,228} = 2.11, p < 0.0299$; lap 2: $F_{9,228} = 2.66, p < 0.0059$; lap 3: $F_{9,228} = 4.06, p < 0.0001$; table 1 and figure 1).

Examination of MAV achieved for each time interval revealed there was a significant effect of time on MAV ($F_{3,204} = 6.35, p < 0.0004$), while treatment and treatment X time had marginal effects on MAV (treatment: $p = 0.0768$; treatment X time: $p = 0.0553$). This pattern appeared to be driven by a 37% and 33% decrease in sprint speed in the highest treatment group compared to control lizards at 4 and 24 hours after exposure to carbaryl, respectively (data not shown). However, when I examined MAV of each lap seperately I found there was a significant effect of both treatment and time for both lap 1 (treatment: $F_{3,66} = 3.75, p = 0.0149$; time: $F_{3,201} = 8.45, p < 0.0001$) and lap 2 (treatment: $F_{3,66} = 5.26, p = 0.0028$; time: $F_{3,201} = 3.94, p = 0.0095$), but no treatment by time interaction (lap 1: $p = 0.3460$; lap 2: $p = 0.3950$; table 2). In both laps, treatment
and time interval effects appeared to be driven by a decrease (29 - 55%) in MAV in the highest treatment group compared to controls at 4 and 24 hrs after dosing (see figure 2).

Both the number of lizards who fell and refused to traverse the arboreal substrate at least once during the arboreal experiment was significantly different among treatments (Overall model; refusals: \( p = 0.0035 \); falls: \( p < 0.0001 \); figure 3). Only 10% of gavage control lizards fell or refused in the arboreal experiment compared to 55 and 85% of lizards in the highest treatment group. Also, lizards in the 25 \( \mu \)g/g treatment group were 2.5X more likely to fall than lizards in the control group.

**Discussion**

Acute exposure to carbaryl resulted in a decrease in lizard sprint speed in both the arboreal and terrestrial setting, but only at the highest dose concentration (250 \( \mu \)g/g). The reduction in sprint speed in the highest treatment group compared to controls at 4 and 24 hrs after dosing in both the terrestrial and arboreal setting was more pronounced in later laps, from no difference in lap 1 to 29% and 30% in lap 3 (terrestrial) and from 37% and 29% in lap 1 to 42% and 55% in lap 2 (arboreal), suggesting that carbaryl affected endurance of lizards (figures 1 and 2). Contrary to my prediction, sprint speed did not decrease in a dose-dependent manner. Instead, there appeared to be a slight stimulatory effect of carbaryl on sprint speed at the two lower dose concentrations, but only in the terrestrial setting. Individuals in these treatment groups exhibited 17-33% increases in sprint speed compared to controls within 24 hrs of treatment administration (figure 1). Although degraded performance only occurred at the highest dose concentration, which may not be frequently encountered in the field, very little information exists concerning pesticide residues on insects after pesticide application. More studies examining the relationship between pesticide application and insect residues are needed to better predict pesticide concentrations insectivorous wildlife are likely to encounter.

As predicted, performance in the arboreal setting appeared to be more challenging than terrestrial locomotion, and as a consequence, arboreal performance also proved to be a more sensitive indicator of carbaryl exposure. Maximum velocity achieved during the arboreal sprint trials was ~50% lower than maximum velocity achieved in the terrestrial experiment. Whereas carbaryl induced reductions in sprint velocity ranged between 13 -
30% in the terrestrial setting, reductions in the arboreal setting were twice as pronounced (29 - 55%). Furthermore, I noted that lizards in the highest treatment group were 4.5X more likely to refuse to traverse the arboreal substrate, and 7.5X more likely to fall than controls during arboreal sprint trials (figure 3). Interestingly, there was also a significant increase in the percentage of individuals that fell in the 25 μg/g (35%) compared to controls (10%). This was the only instance in which carbaryl had a significant negative impact on performance in one of the lower treatment groups. These effects were not captured when examining terrestrial locomotor performance, but could be of importance to *S. occidentalis* because they regularly utilize arboreal substrates for movement and prey capture (Sinervo and Losos 1991; Schlesinger et al. 1993).

Recently, Holem et al. (2006) examined the effects of malathion, an organophosphate (OP), on sprint speed in *S. occidentalis*. Interestingly, the authors found that exposure to 200 μg/g of malathion resulted in a 23% increase in sprint speed that remained elevated for up to 13 days after dosing, even though 70% of lizards in this dose group exhibited clinical signs of AChE-inhibiting pesticide poisoning (e.g., body/limb tremors, twitching) and 20% mortality. Because carbaryl and malathion are both AChE-inhibiting pesticides and have similar modes of toxicity (Hill 1995), one would expect they would have comparable effects on locomotor performance in the same species. However, this was not the case. In my study, lizards in the highest carbaryl dose group exhibited not only clinical signs of exposure to an AChE-inhibiting pesticide (58%), but also a significant reduction in sprint speed. The disparity in responses to carbaryl and malathion may result from peripheral versus central effects of inhibition of AChE. Furthermore, the difference in effects of these two pesticides on locomotor performance in *S. occidentalis* emphasizes that generalizations should be drawn cautiously about the effects of AChE-inhibiting pesticides on whole-animal responses.

The decrease in sprint speed in my highest dose group is consistent with two studies that reported carbaryl degraded swimming performance in several species of semi-aquatic snakes (Hopkins et al. 2005b; Hopkins and Winne 2006). Similar to my study, the authors also noted that animals appear to recover within 96 hours of exposure. Hopkins et al. (2005b) noted that the effects of carbaryl on swim speed were more pronounced as swim distance increased, similar to what was observed in the highest treatment group in
my study. This effect could be of great ecological relevance to behaviors that require stamina, such as extended bouts of combat observed in male *Anolis* lizards during the breeding season (Jenssen et al. 1995, 2000). However, neither of the snake swimming performance studies observed an increase in swimming speed at the lower dose concentrations examined. Several studies examining the effects of carbofuran, another carbamate, on swimming behavior in goldfish observed an increase in burst swimming speed at low concentrations (Saglio et al. 1996; Bretauad et al. 2001; Bretauad et al. 2002). Other studies have noted an increase in activity several hours after exposure to an AChE-inhibiting pesticide (Hart 1993; Timofeeva and Gordon 2002) and another study noted an increase in sprint speed and distance in tadpoles 24 hrs after exposure to carbaryl (Bridges 1997). However, Bridges (1997) and Timofeeva and Gordon (2002) reported a subsequent decrease in activity within these same treatment groups at later time periods which was not observed in my study. A similar effect was also noted in red-winged blackbirds in which activity levels increased with low doses of dimethoate, an OP, but decreased at higher dose concentrations (Brunet et al. 1997). It is possible that an increase in fasciculation which could account for the increase in sprint speed at low dose concentrations of AChE-inhibiting pesticides (ASTDR 2003). Taken together, the results of my study and previous studies suggest that complex dose-response curves may exist for behavior and performance in a variety of fish and wildlife and are worthy of future study.

My results suggest that carbaryl at high dose concentrations compromise behaviors involved with such critical processes as predator avoidance and prey capture. It also appears, based on the results of my study and Holem et al. (2006), that sprint speed in an arboreal setting is a more sensitive indicator of acute AChE-inhibiting pesticide poisoning than terrestrial locomotor performance. Decreases in sprint speed were greater in the arboreal setting than in the terrestrial setting, and effects such as refusal to sprint falling could be important consequences of pesticide exposure that are not apparent in the terrestrial setting. Of particular importance is the finding that similar pesticide compounds with similar modes of toxicity can produce such contrasting results on sprint performance in *S. occidentalis*, indicating the importance of future studies of multiple AChE-inhibiting pesticides on whole-organism responses.
Table 1. Results of repeated measures ANCOVA for the effects of carbaryl treatment on maximum terrestrial velocity (SVL as a covariate) per lap in *Sceloporus occidentalis* before, 4, 24 and 96 hrs after oral gavage with carbaryl.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Effect</th>
<th>Num df</th>
<th>Den df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTV lap 1</td>
<td>Treatment</td>
<td>3</td>
<td>75</td>
<td>1.75</td>
<td>0.164</td>
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<tr>
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<td>Time</td>
<td>3</td>
<td>228</td>
<td>7.14</td>
<td>0.001</td>
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<tr>
<td></td>
<td>SVL</td>
<td>1</td>
<td>75</td>
<td>0.67</td>
<td>0.416</td>
</tr>
<tr>
<td></td>
<td>Treatment X time</td>
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<td>228</td>
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<td>0.030</td>
</tr>
<tr>
<td>MTV lap 2</td>
<td>Treatment</td>
<td>3</td>
<td>75</td>
<td>1.11</td>
<td>0.351</td>
</tr>
<tr>
<td></td>
<td>Time</td>
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<td>0.926</td>
</tr>
<tr>
<td></td>
<td>Treatment X time</td>
<td>9</td>
<td>228</td>
<td>2.66</td>
<td>0.006</td>
</tr>
<tr>
<td>MTV lap 3</td>
<td>Treatment</td>
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<td>74</td>
<td>3.62</td>
<td>0.017</td>
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<td></td>
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<td>13.37</td>
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<td>Treatment X time</td>
<td>9</td>
<td>228</td>
<td>4.06</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*The mixed procedure was performed using SAS (Proc Mixed). Sample size = 10 for each treatment for each sex for all variables.*
Table 2. Results of repeated measures ANCOVA for the effects of carbaryl treatment on maximum arboreal velocity (SVL as a covariate) per lap in *Sceloporus occidentalis* before, 4, 24 and 96 hrs after oral gavage with carbaryl.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Effect</th>
<th>Num df</th>
<th>Den df</th>
<th>$F$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAV lap 1</td>
<td>Treatment</td>
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<td>66</td>
<td>3.75</td>
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<td>Time</td>
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<td>201</td>
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<td></td>
<td>Treatment X time</td>
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<tr>
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<td>Treatment</td>
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<td>201</td>
<td>3.94</td>
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<td>66</td>
<td>1.26</td>
<td>0.266</td>
</tr>
<tr>
<td></td>
<td>Treatment X time</td>
<td>9</td>
<td>201</td>
<td>1.06</td>
<td>0.395</td>
</tr>
</tbody>
</table>

The mixed procedure was performed using SAS. Individuals that refused to run were not included in statistical analyses. Sample sizes varied in statistical models.

**Lap 1:** Sample size = gavage: 9 females, 9 males; 2.5 µg/g: 8 females, 10 males; 25 µg/g: 9 females, 10 males; 250 µg/g: 6 females, 10 males.

**Lap 2:** Sample size = gavage: 9 females, 9 males; 2.5 µg/g: 6 females, 11 males; 25 µg/g: 9 females, 9 males; 250 µg/g: 1 female, 8 males.
Figure 1: Maximum terrestrial velocity achieved by *S. occidentalis* for each of three laps over a 2.3 m track before and 4, 24, and 96 hrs after oral administration of carbaryl. Error bars are ±1 standard error of the mean. N = 10 per sex per treatment group for each lap except for the highest dose group in lap 3 which includes 9 females and 10 males. Sample sizes varied for lap 3 because one female refused to sprint during the 24 hr time point.
Figure 2: Maximum arboreal velocity achieved by *S. occidentalis* for each of two laps over a 1.2 m dowel rod before and 4, 24, and 96 hrs after oral administration of carbaryl. Error bars are ±1 standard error of the mean. Sample sizes varied because some lizards refused to sprint during one or more time intervals.

Lap 1: N = 9 females and 9 males for the gavage control group; 8 females and 10 males for the 2.5 µg/g group; 9 females and 10 males for the 25 µg/g group; and 6 females and 10 males for the 250 µg/g.

Lap 2: N = 9 females and 9 males for the gavage control group; 6 females and 11 males for the 2.5 µg/g group; 9 females and 9 males for the 25 µg/g group; and 1 females and 8 males for the 250 µg/g
Figure 3: Percentage of individuals that refused to sprint across, or fell off of a 1.2 m arboreal track at least once during the course of the experiment. N = 10 lizards per sex per treatment group for all treatments except the 2.5 µg/g group which contained 10 females and 11 males.
Chapter 2

Energy acquisition and allocation in an ectothermic predator exposed to a common environmental stressor.
Abstract
Stressors are commonly encountered by organisms and often prove to be energetically costly. Certain stressors can simultaneously affect multiple components of an animal’s energy budget and can either exacerbate energetic costs to the individual or offset one another. Here I used a commonly encountered stressor, the pesticide carbaryl, to examine the complex effects that acute environmental disturbances can have on energy expenditure, allocation, and acquisition, important processes that influence growth and reproduction. After exposing lizards (Sceloporus occidentalis) to carbaryl, I measured their metabolism over a 48 hr period and assessed their food consumption over 96 hrs. I found no difference in total energy expenditure among treatment groups, but lizards exposed to the highest dose of carbaryl allocated energy differently than other groups. Compared to controls, these lizards exhibited a 16 – 30 % increase in standard metabolic rate (SMR), that was offset by a 45 – 58 % decrease in additional energy expenditures. Lizards in the highest dose group also exhibited a 30 – 34 % decrease in energy acquisition compared to controls. The net result was a 1.83 kJ decrease in energy assimilation, equivalent to 5 times their daily SMR requirements. My results indicate that energetic consequences of stressors may result in complex energetic trade-offs, and I emphasize the need to simultaneously examine the effect of stressors on multiple portions of an animal’s energy budget.

Introduction
Throughout the life of an animal, assimilated energy is allocated towards two competing demands, maintenance and production (Congdon et al. 1982, 2001; McNab 2002). Maintenance costs are essential for the continuity of life and include both standard maintenance (i.e., basal energy costs for living) and activity maintenance (e.g., energy necessary for foraging, circadian rhythms, and digestion), whereas energy allocated towards production supports important life history processes such as growth and reproduction (Congdon et al. 1982). For many organisms, a large proportion of assimilated energy is allocated towards maintenance costs, but substantially less is allocated towards production. For example, in reptiles, approximately 80 % of their energy budget is comprised of maintenance costs, whereas production accounts for the
remaining 20 % (Congdon et al. 1982). Even small increases in maintenance can result in proportionately large decreases in the energy available for growth and reproduction. During times of limited energy availability, energetic costs necessary for living are met, while energy allocated towards high levels of activity and production are often restricted (Lucas 1996; McNab 2002).

Stress, a phenomenon all animals regularly experience, is one factor that can be energetically costly for organisms. McEwen and Wingfield (2003) suggested that stressors increase the energetic burden to the animal, and when this burden exceeds the energy an animal can acquire from the environment, the deleterious effects of stress become apparent. Stressors can be energetically costly for a variety of reasons, including the deployment of processes that protect against stressors (e.g., synthesis of heat-shock proteins; Krebs and Loeschcke 1994), or rid the body of a stressor (e.g., metabolizing and excreting contaminants; Calow 1991), and changes in stress hormone concentrations (Morgan and Iwama 1996). Stressors may also decrease energy assimilation efficiency (Baird et al. 1990), alter the accessibility of energy in the environment (Hopkins et al. 2004a), and influence energy expenditures associated with activity (Congdon et al. 2001).

An extensive number of studies have addressed the energetic cost of both natural (e.g., Barton and Schreck 1987; Christiansen et al. 1991; duPreez et al. 1996; Davis and Schreck 1997; Iwama et al. 1997; Dalla Via et al. 1998; Harris et al. 1998; Sloman et al. 2000; Ricklefs and Williams 2003) and anthropogenic (Rowe et al. 1998, 2001; Hopkins et al. 1999, 2002; Bain et al. 2004) stressors, however, the impact of the stressor on energy expenditure varies greatly among species and types of stressors. Calow (1991) suggested that the difference in the energetic costs of stressors may result from the effects these stimuli have on different biological processes. For example, some processes may be downregulated in response to a stressor, while others might be upregulated, serving to offset energetic costs in some instances and exacerbating costs in others. However, this conceptual framework has rarely been tested directly. Hopkins et al. (2002) noted that benthic fish with restricted access to food resources exhibited the typical hypometabolic response observed in animals facing food deprivation (Wang et al. 2006). However, when food-deprived fish were exposed to an additional stressor (metal-contaminated sediments), the fish failed to reduce their metabolism, which resulted in reduced body
condition and survivorship compared to fish in reference conditions (Hopkins et al. 2002). The author’s suggested that tissue repair and/or upregulation of physiological processes such as metabolism and excretion of contaminants could account for the apparent inability of contaminant-exposed individuals to restrict their energy usage during times of reduced resource abundance. In another example, Barber et al. (1990) expected to find an increase in O$_2$ consumption in *Daphnia magna* after exposure to cadmium and dicholoranaline because of evidence for deployment of energetically costly stress-resisting mechanisms after exposure to these contaminants (Baird et al. 1990). Although there was no significant change in total O$_2$ consumption, the authors noted a reduction in food intake and inferred that a rise in basal metabolism most likely did occur but was offset by a decrease in metabolic costs associated with consuming and digesting food (Barber et al. 1990). The latter two studies suggest that patterns of energy expenditure, allocation, and acquisition under stressful conditions can be complex and that simultaneous monitoring of several parameters related to energy use can provide insight into the ultimate energetic consequences of a stressor to an individual.

To further probe the complex effects of stressors on animals’ energy budgets, I chose to study the chemical carbaryl, one of the most widely used non-commercial insecticides which also has an extremely well-defined mode of toxicity. Carbaryl inhibits the breakdown of acetylcholine (ACh), a neurotransmitter found at neuromuscular junctions, by deactivating the enzyme acetylcholinesterase (AChE) (Fukuto 1990). It is among the least toxic pesticides in its class and thus is used for a wide variety of applications (Hill 2003). According to the US EPA Pesticide Industry Sales and Usage 2000-2001 Market Report (Kiely 2004), carbaryl was ranked as the sixth most commonly used conventional pesticide in the home and garden market. Because carbaryl and other AChE-inhibiting pesticides are very short-lived in the environment (Half-life in soil 8 – 18 days; Nkedi-Kizza and Brown 1998), pulse exposures representing acutely stressful events are expected to be more ecologically relevant encounters than low-level chronic exposure typical of some other environmental pollutants (e.g., metals).

In the present study I determined the effects of this commonly encountered environmental stressor on energy acquisition, expenditure, and allocation in a lizard. I quantified total energy consumption and how much of this energy was consumed to
support basal metabolism for 48 hrs after exposure to a carbaryl. I also examined whether exposure to the stressor would alter energy acquisition because I hypothesized that a compensatory increase in food consumption could theoretically alleviate energetic costs of the stressor. Alternatively, a decrease in energy acquisition could exacerbate any energetic costs of the stressor and further compromise an individual’s energy balance. Measuring multiple metabolic parameters and energy acquisition allowed us to explore the energetic tradeoffs that potentially occur in animals experiencing stress.

**Materials and Methods**

*Fence lizard natural history and husbandry*

*Sceloporus* lizards belong to the family Phrynosomatidae which accounts for more than 30% of all lizard species in the United States. Some *Sceloporus* serve as good study organisms because their entire lifecycle is manageable in the laboratory, and a great deal is known about their ecology, physiology, performance and life history (e.g., Garland et al. 1990; Sinervo 1990; Angilletta et al. 2002; Talent et al. 2002; Roe et al. 2005). The species used in this study, the western fence lizard (*Sceloporus occidentalis*), ranges from Mexico to Canada between the California coast and western Utah, USA. The original parental stock of my laboratory subjects originated from a population in the grasslands of the San Joaquin Valley, CA, USA. Most females from this population reach sexual maturity within one year under ad libitum feeding conditions in the laboratory, and lay 3 – 6 clutches of 8 – 15 eggs per year. This population of western fence lizards does especially well under laboratory conditions and has been identified as a good candidate for use as a laboratory reptile model (Talent et al. 2002; Hopkins et al. 2005b). Juvenile western fence lizards were shipped to The Savannah River Ecology Laboratory (SREL) from a breeding colony at Oklahoma State University. Lizards husbandry was identical to Hopkins et al., (2005) with the following exceptions: a 10:14 (light: dark) photoperiod, a daytime temperature gradient of ~28-40°C, and a diet consisting of 4 crickets (~1.5 cm each) per day. A total of 117 lizards were used in the following experiments and no lizard exposed to carbaryl was used in additional experiments. However, some control lizards were used in both experiments to reduce the number of lizards sacrificed during my studies. Control lizards used in both experiments
were given at least two weeks to recover from the first experiment and then were evenly distributed among the three pesticide treatment groups in the second experiment. Experimental procedures were approved by the University of Georgia IACUC (A2004-10049-0).

Experiment I: Energy Expenditure and Allocation

In the first experiment I determined the effect of carbaryl on lizard metabolism. I simultaneously measured both the volume of oxygen consumed \((V_O2)\) and volume of carbon dioxide produced \((V_CO2)\) every 1.18 hrs over a 48 hr period for a total of 38 – 39 measurements using a computer-controlled closed circuit respirometer (Micro-Oxymax, Columbus Instruments Columbus, OH) following methods similar to Hopkins et al. (2004) and Roe et al (2005). Lizards were post-absorptive (i.e., fasted 48 hrs) during all respirometry trials. After fasting lizards were assigned to one of five treatment groups; control, gavage control, 2.5 µg/g, 25 µg/g, or 250 µg/g carbaryl. Lizards were then weighed and administered their respective treatment. After treatment administration lizards were placed in individual respirometry chambers (1000 ml Erlenmeyer flask) covered with paper to visually isolate them from external stimuli; the respirometry chambers were then placed in an environmental chamber at 30°C for the duration of the trial. All trials were conducted between March and August of 2004. Each treatment group was comprised of six lizards per sex. However, a male in the highest treatment group died 41 hours after being exposed to carbaryl. To equalize sample sizes among treatments, I replaced this individual with another male.

Carbaryl was administered to lizards via oral gavage using an Eppendorf micro-pipette (2 – 20 µl). The 2.5 and 25 µg/g solutions were made within one hour of administration by diluting the 22.5% formulation of carbaryl (Sevin®; Garden Tech, Lexington, KY, USA) with water and then vortexing the solution for one minute. Lizards in the 250µg/g treatment group received an undiluted dose of the 22.5% Sevin® formulation and lizards in the oral gavage treatment group received a comparable volume of water. Control lizards were not manipulated. All lizards used in the respirometry trials weighed between 6.3 – 9.1 g, therefore dose volumes ranged between 6.5 – 9.4 µL.
Although contaminated insects are an important route of exposure to pesticides for insectivorous vertebrates inhabiting areas that receive pesticide application, very little data exists on invertebrate pesticide residues. Therefore, it is difficult to predict actual concentrations lizards are likely to encounter in the wild. In the only study to directly quantify carbaryl residues of terrestrial invertebrates, Fair et al. (1995), showed that grasshoppers had mean residues of 17 µg/g two days following rangeland application of 0.5 kg active ingredient/ha. Using this insect residue data and carbaryl application rates, which can vary from 1.12 to 22.42 kg active ingredient/ha (EPA 2004) I estimated that a 10 g lizard consuming 1 g of prey could ingest dose concentrations ranging between 3.9 – 78.5 µg/g 2 days following carbaryl application. Factoring the short half-life of carbaryl into my estimates, I selected three doses that fully encompass the range of concentrations that lizards could encounter in the environment.

Using the respirometry data collected I quantified three metabolic parameters: 1) total $V_O_2$ and $V_CO_2$, 2) $V_O_2$ and $V_CO_2$ to support standard metabolic rate (SMR), and 3) the amount of gas exchanged to support metabolic activity exceeding SMR (e.g., spontaneous activity, circadian rhythms; hereafter, referred to as additional $V_O_2$ and $V_CO_2$). Total $V_O_2$ and $V_CO_2$ were estimated as the integral (i.e., area under the curve) of respiration ($V_O_2$ and $V_CO_2$) over the entire respirometry trial. Because SMR is the metabolic rate of a resting, post-absorptive ectotherm at a specified temperature during the inactive phase of its circadian cycle (Bennett and Dawson 1976), I estimated SMR as the lowest quartile value from each individual’s respiratory profile, a procedure that removes peaks in respiration associated with activity and circadian rhythms. Other studies on reptiles have been successful at estimating SMR using similar methods (Hopkins et al. 2004). $V_O_2$ and $V_CO_2$ to support SMR were estimated as the integral of SMR. Additional $V_O_2$ and $V_CO_2$ were estimated by subtracting the integral of SMR from the total $V_O_2$ and $V_CO_2$. Total respiration, additional respiration, and respiration to support SMR were then converted to units of energy expended (1 ml O$_2$ consumed = 19.8 J; Secor and Diamond 1995). I also determined respiratory quotients (RQ: the ratio of CO$_2$ produced to O$_2$ consumed) for each lizard to allow inference about the substrates used for aerobic catabolism (Withers 1992).
Experiment II: Energy Acquisition

In the second experiment, I sought to determine whether exposure to carbaryl affected food consumption. After fasting lizards for 48 hrs, I then assigned them to one of the five aforementioned treatment groups, their masses were recorded, and their respective treatment was administered via oral gavage. During feeding trials, lizards were housed individually in a 52 X 36 X 18 cm plastic cage with a screen lid arranged in a rack system. At 24 and 96 hrs post dose administration (repeated measures on individuals), each lizard was offered 10 crickets weighing (in total) 15% of the lizard’s body mass. Twenty-four hours following each feeding trial, all remaining crickets were removed, counted, and weighed. Lizards were not fed during the 48 hrs between the 24 and 96 hrs feeding trials. Lizard masses in this experiment ranged between 6.1 – 9.5 g and dose volumes ranged between 6.2 – 9.8 µl. Treatment groups were comprised of eight lizards per sex. Feeding trials were conducted between March – May of 2004.

To determine the energy content of prey items, three composite samples of crickets were lyophilized and ground into a powder. Energy content of homogenized samples was determined using an adiabatic bomb calorimeter (Parr instrument Co., Moline IL, USA) at the Poultry Science Research Laboratory of the University of Georgia.

Statistical Analyses

I examined the influence of treatment on total respiration, additional respiration, and respiration to support SMR (V_{O2} and V_{CO2} for each parameter) using a multivariate analysis of variance (MANOVA). Wilks’ lambda statistical values were used to assess statistical significance. All respiratory variables and mass were log_{10}-transformed to better approximate assumptions of the model. In the model, I considered treatment and sex as independent variables and log_{10}-transformed mass as the covariate. The initial model included all interaction terms; insignificant interactions were dropped in subsequent iterations of the model. Initial models indicated there was no difference in respiration between males and females, and therefore sexes were combined for statistical analyses. Individual ANCOVAs for each dependent variable were also examined to determine which variables contributed to significant effects in the multivariate model.
Because the variance associated with RQ values was not equivalent among treatments, I compared RQs among treatments using a mixed model approach to ANCOVA (SAS PROC MIXED). Log_{10}-transformed mass was treated as a covariate and RQ values were arc-sin square root transformed to better approximate a normal distribution. Because RQ was not independent of the other respiratory variables, I applied a sequential bonferroni adjustment to account for multiple, non-independent comparisons.

To examine the influence of treatment on food consumption I conducted a repeated measures ANOVA using a mixed model approach (SAS PROC MIXED). The percentage (arc-sin transformed) of a lizard’s body mass consumed in crickets was treated as the dependent variable, treatment and sex were treated as independent variables, and time was the repeated variable. Initial models indicated there was no difference in respiration between males and females (p ≥ 0.39), and therefore sexes were combined for statistical analyses. Because I was interested in whether lizards would recover from exposure over time, I also included the interaction between treatment and time in the model.

Results

Oxygen consumption and carbon dioxide production of lizards were influenced by treatment (Wilks lambda = 0.46; F_{24,172} = 1.8; p = 0.019; Figure 1), but examination of individual ANCOVA’s for each respiratory parameter revealed that treatment effects were dependent upon which parameter was considered (Figure 2 and 3). Treatment had no effect on total VO_{2} or V_{CO2} (p > 0.582). However, VO_{2} and V_{CO2} to support SMR were significantly affected by treatment, namely a 16-30% increase in gas exchange to support SMR (equivalent to 0.11-0.14 kJ) in the highest treatment group compared to the two control groups (VO_{2}: F = 2.9, p = 0.029; V_{CO2}: F = 2.9, p = 0.030). Furthermore, treatment had a significant affect on additional VO_{2} (F = 2.6, p = 0.047) and a marginal affect on additional V_{CO2} (p = 0.061). This resulted from a 45 – 58 % decrease in additional VO_{2} and V_{CO2} in the highest treatment group compared to controls (equivalent to 0.15 – 0.28 kJ). However, treatment had no effect on RQ (range of mean RQs = 0.77 - 0.81; p = 0.615); suggesting that lizards in all treatment groups were metabolizing similar substrates to meet their energy requirements.
There was a significant effect of treatment on food consumption, but this effect was dependent upon time (treatment: $F_{4,75}=2.94, p=0.03$; time: $F_{1,75}=6.53, p=0.01$; treatment X time: $F_{4,75}=7.01, p < 0.001$). The highest treatment group exhibited a 30 – 34 % decrease in food consumption compared to controls, but recuperated within 96 hrs (Figure 4). Energy content of crickets was $5.8 \pm 0.01$ kJ per gram of cricket.

**Discussion**
My study is among the first to demonstrate that stress-induced changes in energy allocation to multiple portions of the energy budget can offset one another. I found that while total energy expended did not differ among treatments, lizards exposed to the highest dose of carbaryl allocated energy differently than lizards in other groups (Figure 3). Specifically I found that lizards in the highest dose group allocated 16 – 30% more energy towards SMR than control groups (Figure 2), but 45 – 58% less energy towards additional energy expenditures (Figure 2). Alterations in energy allocation after acute exposure to carbaryl were sustained for at least 44 hrs (Figure 1). In addition to altered energy allocation, lizards in the highest dose group also exhibited a 30 – 34 % decrease in food consumption during the 24 hour feeding trial compared to controls. Thus, although changes in energy allocation were compensatory, lizards still experienced energetic consequences from exposure to the stressor due to decreased energy acquisition.

An increase in SMR and an apparent decrease in spontaneous activity appear to be the two primary effects that offset one another. Processes such as the release of stress hormones (Wendelaar-Bonga 1997), breakdown and excretion of contaminants (Calow 1991), the effects of carbaryl on muscle fasciculation, and perhaps the direct effects of AChE-inhibitors on the respiratory system (Grue et al. 1991) can be energetically costly and may have contributed to the increase in SMR observed in lizards in the highest dose group. The temporal patterns in gas exchange (Figure 1) suggest that decreased spontaneous activity resulted in the decrease in additional energy expenditure; this is consistent with the results of two other studies that noted decreased activity in leopard frog tadpoles (Bridges 1997) and red-winged blackbirds (Brunet et al. 1997) after exposure to AChE-inhibitors. Taken together, my findings support the idea that
environmental stressors simultaneously can have complex and differing effects on the various components of metabolism (Calow 1991).

A substantial amount of research exists on the effects of stress on appetite and food intake. The bulk of which indicates that severe stress reduces food ingestion (Carr 2002), thereby negatively impacting an individual’s energy budget through decreased energy acquisition. In my study, I found that although energy allocations were offsetting, lizards exposed to the highest dose of carbaryl would still experience negative energetic consequences compared to controls due to a 30 – 34 % reduction in food consumption (Figure 4). For a ten gram lizard, this period of prandial quiescence would result in 440 mg less food being consumed compared to a similarly sized control lizard. Assuming that 72 % of the energy in the ingested food would be assimilated (Angilletta 2001), I estimate that lizards exposed to high concentrations of carbaryl could experience a 1.837 kJ/day decrease in energy assimilation compared to controls. This net decrease would be significant, exceeding my estimates of the daily standard maintenance requirements of lizards (0.35 kJ/day) by 5-fold. It is worth noting that energy allocation and clinical symptoms (e.g., muscle twitching) were altered for almost 48 hrs. Because I did not continuously monitor food consumption for the entire 48 hrs period, it is possible that my calculations are an underestimate of the effect of carbaryl on energy acquisition (Figures 1 and 4).

Although changes in energy allocation and acquisition were short-lived, which is consistent with the biochemical properties of carbaryl (Fukuto 1990) and the duration of its biological effects (Hopkins et al. 2005a; Hopkins and Winne 2006; DuRant et al. InPress), wildlife are exposed to this and many other stressors in repeated pulses over each active season (Davidson et al. 2002). In addition, stressors that are persistent in the environment, such as social stress, disease, and invasive species, could result in extended periods of altered energy expenditure and/or energetic trade-offs. In fact, such extended alterations in metabolism have been noted in animals exposed to heavy metals, a persistent environmental stressor (Hopkins et al. 1999; Rowe et al. 2001). As suggested by McEwen and Wingfield (2003) animals experiencing negative energy balance may enter an emergency life history stage to regain favorable energy relations. Serious energetic trade-offs and periods of elevated energy expenditure could result in the
triggering of an emergency life history stage, and may cause the individual to forego important life history events, such as reproduction.

Even though I observed no net change in total energy expenditure, my results indicate that changes in energy allocation associated with stress can be dynamic and complex. All else being equal, an increase in energy use for one portion of the energy budget will cause an energy shortfall in others. In most previous studies, an increase in basic maintenance requirements caused by stress (increase in SMR or BMR) has been suggested to detract from the energy available for production (e.g., Hopkins et al. 1999). Here I demonstrate that moderation in activity may be sufficient to offset these costs. However, decreased spontaneous activity also has consequences for lizards because it could alter susceptibility to predation (Weis et al. 2001), decrease foraging, and possibly decrease digestive performance given the importance of basking to lizard digestion (Angilletta 2001). In the end, sustained changes in energy allocation and acquisition could ultimately result in decreased growth and reproduction. However, additional studies on repeated acute stress or sustained stress are needed to understand the implications of these effects on the production portion of an individual’s energy budget.
Figure 1. Mean (± 1SE) oxygen consumption (A) rates (ml/h) and carbon dioxide production (B) rates in western fence lizards (*Sceloporus occidentalis*, 6.3 – 9.1 g) at 30°C acutely exposed to 250 µg/g carbaryl (closed circles) compared to controls (open circles) over a 48 hr period. The dashed line and solid line represent SMR for lizards exposed to 250 µg/g carbaryl and control lizards, respectively. N=6 lizards/sex/treatment.
Figure 2. Patterns of oxygen consumption (A) (ml) and carbon dioxide production (B) among western fence lizards (Sceloporus occidentalis) acutely exposed to varying concentrations of carbaryl. Total oxygen consumed over a 48 hr period (Total) following carbaryl administration is partitioned into oxygen consumed to support standard metabolic rate (SMR) and that consumed to support additional energy expenses (Additional). Data are presented as means ± 1SE. N= 6 lizards/sex/treatment.
Figure 3. Summary of energy consumption (kJ) patterns among western fence lizards (*Sceloporus occidentalis*) acutely exposed to varying concentrations of carbaryl. Total oxygen consumed is allocated to either standard metabolic rate (solid portion of bar) or to support additional energy expenses (open portion). *N*= 6 lizards/sex/treatment.
Figure 4. Patterns of food consumption, calculated as the percentage of the individual’s body mass it consumed in crickets, in western fence lizards (*Sceloporus occidentalis*) after acute exposure to varying concentrations of carbaryl. Lizards were offered 15% of their body mass during each trial. Hatched and open bars represent 24 and 96 hr feed trials, respectively. Data are presented as means ± 1SE. *N* = 8 lizards/sex/treatment.
Conclusions

My study is one of the few large-scale assessments of dose-response relationships for any contaminant in a reptile. Because so little is known about the impact of contaminants on reptiles, studies examining impacts relevant to the animal’s fitness are of utmost importance. In this study, I found that a single acute exposure to a commonly-used pesticide reduces locomotor performance, increases basal metabolism, reduces additional energy expenditure, and decreases energy acquisition in a widespread lizard. Taken together, the results of my study indicate that carbaryl can negatively impact traits important to survival and reproduction in lizards. Fortunately, the negative effects of the pesticides on the fitness-related traits I examined were only detected at the highest dose concentration, except for an increase in the likelihood of a lizard in the 25 μg/g dose group to fall in the arboreal setting compared to controls. Therefore, my observed alterations in energy allocation, energy acquisition, and performance resulting from exposure to carbaryl may represent a worst case scenario. However, the dearth of information on pesticide residue concentrations on invertebrates encountered by free-ranging wildlife introduces considerable uncertainty into my estimates of the upper limits of ecological exposure concentrations. This important knowledge gap needs to be filled not only to benefit risk assessments for lizards, but also for other insectivorous wildlife such as birds and mammals.

My work also lends insight into which whole-organism responses might be most sensitive to AChE-inhibiting pesticide poisoning in lizards. Based on the results of the terrestrial and arboreal locomotor performance experiments I found that behaviors that require greater coordination and balance (i.e., arboreal performance) suffered more than less complex behaviors (i.e., terrestrial performance). Although my work should be corroborated with studies of other lizards and/or pesticides, my findings suggest that terrestrial locomotor performance in lizards may not be a good indicator for AChE-inhibiting pesticide exposure. Conversely, energy allocation and acquisition both rapidly and markedly responded to carbaryl exposure indicating that these traits are sensitive indicators of AChE-inhibiting pesticide poisoning. The importance of these traits to growth and reproduction make them especially important to monitor when investigating
AChE-inhibiting pesticides that have longer-lasting effects than carbaryl, such as organophosphates.

Although acute exposure to AChE-inhibiting compounds are ecologically relevant due to their short half-life in the environment, animals are often chronically or repeatedly exposed to pulses of these compounds over relatively short time periods (e.g., days to weeks; Davidson et al. 2002; EPA 2004). Bridges (2000) found that tadpoles chronically exposed (throughout development) to carbaryl at concentrations an order of magnitude less than expected environmental concentrations exhibited a dramatic increase in mortality, greater than predicted by LC50s, and high malformation rates. An additional study (Anderson et al. 2006) noted that Daphnia subjected to repeated pulse exposures of AChE-inhibitors exhibited increased mortality compared to animals exposed to a single exposure.

Though my research focused on effects of pesticides on individuals, but it is also important to note that AChE-inhibiting pesticides not only impact individuals, but entire communities in the field (Boone and Bridges 2003; Mills and Semlitch 2004; Metts et al. 2005). In addition to the direct effects pesticide exposure can have on an animal, exposed wildlife must often cope with the indirect effects of the pesticide as well (e.g., reduced food abundance; Mills and Semlitch 2004, Metts et al. 2005). In fact, it has been suggested that the indirect effects of contaminants may be more important to the individual’s health than direct effects of the pesticide (Boone and Bridges 2003; Fleeger et al. 2003). Because of the known importance of these effects on wildlife the direct effects caused by exposure to an AChE-inhibiting pesticide I noted may constitute only a fraction of the total cost of exposure for an individual. Furthermore, animals in the wild typically cope with additional stressors that are unrelated to pesticide exposure such as parasites, invasive species, or habitat destruction. Therefore, the possibility exists that exposure to AChE-inhibiting pesticides may make animals more susceptible to additional stressors, or that coping with a stressor like parasite infestation could make an animal more susceptible to pesticides. Thus, I conclude that my laboratory-derived estimates of adverse effect levels are conservative, and probably underestimate effects that would occur in real-world situations.
While the four present experiments helped address the effect of carbaryl on several whole organism responses, it is important that these responses ultimately be linked to effects at the subcellular level. As mentioned earlier, measuring AChE-inhibition is a well-established biomarker, but for this biomarker to be most valuable we must understand what levels of enzyme inhibition cause outcomes that are meaningful to an individual’s fitness. Despite the assumption that AChE-inhibition implies physiological impairment in animals, very few studies have examined this relationship (but see Beauvais et al. 2000; Beauvais et al. 2001; Sandahl et al 2005), and it is unexplored in reptiles. I plan to eventually build on the current study by measuring blood and brain cholinesterase inhibition in *S. occidentalis* after exposure to the same range of dose concentrations of carbaryl. By measuring enzyme inhibition and understanding linkages to effects at the whole organism level, we can greatly improve the effectiveness of this biomarker as an indicator of animal health and relate environmental pesticide exposures to the well-being of lizard populations.

In conclusion, while the use of pesticides has resulted in multiple benefits for crop productivity and human sustenance, their use also poses potential adverse effects on environmental and public health. Included in the cost associated with pesticide use is the impact they have on fish and wildlife populations. To best conserve the quality of the natural world, we must limit the impact pesticides have on the environment. While the use of pesticides has been, and will continue to be, an integral part of modern society, pesticide use must be done in as ecologically responsible manner as possible to reduce the risk of unintended side-effects. To this end, we must continue to investigate potential pesticide-induced effects on the environment. Only in understanding the vast array of effects pesticides have on non-target fish and wildlife will we provide the information necessary to enable managers and regulators to make informed decisions about what pesticides are safest to use and at what rates they can be applied.
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