Physiological performance of Thamnophis hammondii is not affected after eating the toxic frog Xenopus laevis

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PHYSIOLOGICAL PERFORMANCE OF *THAMNOPHIS HAMMONDII* IS NOT AFFECTED
AFTER EATING THE TOXIC FROG *XENOPUS LAEVIS*.

by

C. Drew Foster

**THESIS**

Submitted in partial fulfillment of the requirements for the degree of

**MASTER OF SCIENCE in BIOLOGICAL SCIENCES**

In the Graduate School, Eastern Illinois University

Charleston, Illinois

2004

I hereby recommend that this thesis be accepted as fulfilling this part of the graduate degree cited above

_________________________________________  __________________________________________
Date                                                Thesis Director

_________________________________________  __________________________________________
Date                                                Department/School Head
Exotic species often negatively impact native wildlife. Exotic species might benefit some native species, however, as an additional prey item. Despite posing a serious threat to native aquatic wildlife in California, the toxic African clawed frog (*Xenopus laevis*) provides an additional food source for the two striped garter snake (*Thamnophis hammondii*). Eliciting oral dyskinesia in other snake species, the toxins of *X. laevis* do not affect the behavior of *T. hammondii*. Because there is an increased risk of mortality associated with a compromised locomotor performance, I examined the locomotor performance (sprint speed and endurance) of *T. hammondii* after consuming *X. laevis*. Eleven snakes were used in this study. Each snake crawled down a 2-m long racetrack four times with each replicate separated by 3 min. Subject endurance and sprint speed were measured when subjects were (a) not recently fed, (b) fed sunfish (*Lepomis* spp., a non-toxic prey), and (c) fed *X. laevis*. Contrary to other studies, the presence of a prey item in the gut did not affect sprint speed or endurance. Ingestion of *X. laevis* did not affect either measure of locomotor performance of *T. hammondii*. There was no increased risk of mortality, due to a compromised performance, to *T. hammondii* associated with the predation of *X. laevis*. 
DEDICATION

This thesis is dedicated to my wonderful wife, Jenny. I appreciate her patience with me during the long hours and late nights of working on these research projects. Without her help I could never have accomplished this thesis. Her support and motivation has been a major factor in the completion of my Master’s of Science degree.
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Exotic species

Established populations of exotic species pose various threats to native flora and fauna (Patten and Erickson 2001), and often negatively impact the physical environment and ecological communities (Mack et al. 2000, Pimental 2002, Agrawal and Kotanen 2003). Whether accidentally or deliberately, humans commonly transport exotic species that frequently out-compete or depredate native organisms (Jenkins 1996). Historically, exotic species have been intentionally introduced (1) as game or pets (Lockwood 1999), (2) for landscaping (Cully et al. 2003) or agricultural (Hoddle 2004) purposes, or (3) as attempted biological control agents of a previously introduced and established invasive species (Mungomery 1935, Louda and O’Brien 2002, Hoddle 2004).

Exotic species might negatively impact native species directly through competition, introducing diseases or pathogens (Kiesecker et al. 2001, Blaustein and Kiesecker 2002), hybridizing with native species, and depredating native species (Kats and Ferrer 2003). Exotic species can also affect native species indirectly through the alteration of habitat (Rabenald et al. 1998). The magnitude of these effects can range from an increased pressure on the native species, potentially decreasing its fitness, to its extirpation or extinction (Savidge 1987).

The negative effects that exotic species have on native species often result from a lack of shared evolutionary history between the taxa (Kats and Ferrer 2003). In its new environment, the exotic species often lacks predators. The absence of
predators potentially magnifies the impacts that exotic species have on native species – the exotic species has escaped the guild of predators which were responsible for regulating its population numbers in its natural geographic range (Hoddle 2004). Thus, the exotic species’ population has the potential to grow very rapidly because there are no predators restricting its population numbers. For all of these reasons, introduced species are one of the most serious threats to the survival of native wildlife (Wuerthner 1996), and to the preservation of many species of conservation concern (Yates and Hobbs 1997, Hobbs and Yates 2000, Siverston and Clarke 2000).

Effective management strategies for invasive species are not well understood (Simberloff 2003). Nonetheless, potential introductions of exotic species have been prevented (Parliamentary Commissioner for the Environment 2001). Additionally, established exotic species populations have been successfully eradicated (Myers et al. 2000, Forsyth et al. 2003) and controlled (Simberloff 2002).

For some native species there can be advantages associated with the introduction of a new species. Exotic plant species can provide cover for wildlife or suitable nesting sites for birds (Schmidt and Whelan 1999). Exotic species might also provide an additional food source for some native species (King 2004).

The State of California has a large latitude range and a diverse topography and climate, which provide an array of ecosystems and varied conditions for the native flora and fauna. Unfortunately, these conditions also allow for the successful establishment of many exotic species. In California, there are new discoveries of alien organisms every year (Bury and Luckenbach 1976). One such introduced species occurring in California is the African clawed frog (Pipidae: *Xenopus laevis*).
**Xenopus laevis** natural history

The African clawed frog is an exotic species in many geographic areas, with established populations occurring in the United States, the United Kingdom, and Ascension Island (McCoid and Fritts 1980). In the United States, *X. laevis* has been discovered in Wyoming, Wisconsin, New Mexico, Utah, Florida, Nevada, and Colorado (McCoid and Fritts 1980), and established populations of *X. laevis* occur in Virginia, Arizona, and California (McCoid and Fritts 1993). Arizona, Nevada, Utah, and California currently prohibit the importation of this species (Bury and Luckenbach 1976). Populations of *X. laevis* were initially discovered in California in 1968 (St. Amant et al. 1973) and were most likely established following the intentional release of animals that were no longer needed for their intended laboratory purpose (Kats and Ferrer 2003). Other methods of introduction (e.g., the pet trade, unintentional release, etc.) are possible, however.

Established populations of *X. laevis* pose a potentially serious threat to the native aquatic wildlife (Bury and Luckenbach 1976). This exotic anuran has been described as a voracious, non-specific predator of vertebrates (St. Amant et al. 1973, McCoid and Fritts 1980) and a prolific breeder (Bury and Luckenbach 1976), which makes it an even larger threat to native taxa. Mosquitofish (McCoid and Fritts 1980) and treefrogs (Mahrdt and Knelfer 1972) have become locally extinct in areas of California where the clawed frog occurs. Populations of *X. laevis* in California also pose a threat to the endangered tidewater goby (Eucyclogobius newberryi; Lafferty and Page 1997). The skin secretions of *X. laevis* contain toxins that make it an inedible and undesirable prey item to some species of snakes. An inability of some
snakes to consume *X. laevis* potentially magnifies the threat of this exotic frog (i.e., a lack of population control of the clawed frog might exist if toxins prevent native species from feeding upon it).

**Toxins**

All amphibians have skin secretions, many of which contain toxins. Habermehl (1981) suggested that the evolutionary origin of these toxins was as a defense against bacterial and fungal skin infections. Individuals having their toxins removed died of skin infections within a few days. Toxic skin secretions of amphibians have evolved to a secondary role as an antipredatory mechanism. Amphibian toxins deter many would-be predators (Brodie 1968, Brodie and Brodie 1990, Mobley and Stidham 2000) including many snake species (Garton and Mushinsky 1979, Mori 1989, Brodie et al. 1991, McCallum 1994).

*Xenopus laevis* skin secretions contain the indolealkylamines, serotonin (5-HT) and bufotenidine, and peptides such as cholecystokinin octapeptide (CCK-8), caerulein (CRL), thyrotropin-releasing hormone, and xenopsin (XN; Daly et al. 1987). CCK-8 and CRL (Erspamer and Melchiorri 1973, Zetler 1985, Phillips et al. 1986), 5-HT (Ennis et al. 1981), and XN (Bissette et al. 1986) have neuroleptic properties. Neuroleptics elevate levels of prolactin (Laping and Ramirez 1986), oxytocin (Argiolas et al. 1986), and alphamelanocyte stimulating hormone and adrenocorticotropic hormone (Ferrari et al. 1963, Bertolini and Gessa 1981), all of which have been demonstrated to induce yawning.

Barthalmus and Zielinski (1988) suggested that the suite of toxins found in *X. laevis* acts on the predator through oral absorption. The neuroleptic-like toxins within
The $X.$ laevis toxin complex are the direct cause of the oral dyskinesia (described as yawning and gaping movements; Barthalmus and Zielinski 1988) observed in some snake species when fed $X.$ laevis. The North American natricine $Nerodia$ sipedon exhibited oral dyskinesia when fed $X.$ laevis (Barthalmus and Zielinski 1988). The African water snakes $Lycodon$ morphus rufulus and $L.$ laevissimus, co-occurring with the natural geographic range of $X.$ laevis, also displayed gaping behaviors when fed this toxic frog, but to a lesser extent than the dyskinesia exhibited by $Nerodia$ (Zielinski and Barthalmus 1989). The toxins triggering the observed oral dyskinesia are fast acting (within 30 sec of contact with mucus in $Nerodia$; Barthalmus and Zielinski 1988), often allowing the escape of the frog from the predator (Zielinski and Barthalmus 1989). Despite these observations, introduced populations of $X.$ laevis provide an additional food source for one species of snake native to California (Mahrdt and Knefler 1972, Crayon and Hothem 1998, Ervin and Fisher 2001). The two-striped garter snake (Colubridae: $Thamnophis$ hammondii) co-occurs with established populations of $X.$ laevis and has been observed feeding on this toxic anuran with no observable ill effects (Ervin and Fisher 2001).

$Thamnophis$ hammondii natural history

The genus $Thamnophis$ is a wide-ranging group of about 30 snake species that commonly feed upon annelids, fish, amphibians, reptiles, small birds, and small mammals (Wattiez et al. 1994, Rossman et al. 1996, de Queiroz et al. 2002). Although there are three aquatic specialists ($T.$ melanogaster, $T.$ rufipunctatus, and $T.$ couchi), the majority of Thamnophiine snakes are generalist predators (Rossman et al. 1996).
Some species of the genus *Thamnophis* can tolerate certain toxic amphibian species. *Thamnophis sirtalis*, *T. sauritus*, and *T. proximus*, have all been observed preying upon *Notophthalmus viridescens louisianensis* (Carpenter 1952, McCallum 1994), a species containing tetrodotoxin and toxic peptides and proteins (Daly et al. 1987). *Thamnophis fulvus* is known to feed on *Bolitoglossa rostrata* (Brodie et al. 1991), a species containing serotonin, a biogenic amine (Daly et al. 1987).

*Thamnophis sirtalis* readily feeds on *Taricha granulosa*, although they are not completely resistant to the tetrodotoxin (Brodie and Brodie 1990). *Thamnophis sirtalis* also feeds on *Gastrophryne carolinensis*, although they exhibit some behavioral effects due to the toxins (e.g., gaping, wiping mouth, etc.; Garton and Mushinsky 1979). *Thamnophis hammondii* occurs in sympatry with introduced populations of *X. laevis*, and has been observed feeding on this toxic species (Mahrdt and Knefler 1972, Ervin and Fisher 2001).

*Thamnophis hammondii* is a species of conservation concern in California, although it is common in eastern San Diego County (Jennings and Hayes 1994). Upland populations (approximately 1500 m) of *T. hammondii* can be distinguished from lowland populations (approximately 250 m) in size and diet. Only individuals of *T. hammondii* occurring in lowland populations co-occur with the introduced *X. laevis*, and those individuals occurring in sympatry with this frog are larger in both mass and snout-vent length (SVL; Mullin et al. 2004). The size difference between snakes of upland and lowland areas might be explained by the differences in prey availability (c.f. Lindell 1997) or in the ability of the snakes to shift to eating non-native prey (i.e., *X. laevis*) when the native prey items are less abundant. The difference in snake body
size might result from other factors, however (e.g., differences in ambient
temperatures and length of activity season between the upland and lowland areas).

It is unknown how \textit{T. hammondii} is able to consume \textit{X. laevis}, which is toxic to
some snake species. Evolutionary change is an unlikely mechanism for the resistance
of \textit{T. hammondii} to \textit{X. laevis} toxins unless occurring on a rapid time scale.

Evolutionary change can result from a variety of processes, including natural
selection, gene flow, random genetic drift, and mutation (Stebbins 1977). Interactions
among species are one of the greatest evolutionary forces and a powerful cause of
evolutionary change. The evolutionary arms race (Dawkins and Krebs 1979) fueled
by these interactions describes how predator species respond to the antipredatory
strategies of prey that co-exist with them naturally, and vice versa. \textit{Thamnophis
hammondii} did not historically co-exist with \textit{X. laevis}, but due to the introduction and
establishment of \textit{X. laevis} populations in California, the two species have been
sympatric for approximately 40 years. This brief sympatry might indicate a rapid
evolutionary change following the introduction of the new exotic prey species
(Malhotra and Thorpe 1991). Alternatively, that the two species have co-existed for
only 40 years is likely indicative of an innate resistance to the toxins by \textit{T. hammondii}.

Although \textit{T. hammondii} is able to consume \textit{X. laevis} with no observable
behavioral effects (Ervin and Fisher 2001), no study has examined the effect(s) that
ingesting the frog has on the physiological/locomotor performance of the snake.
Locomotor performance is heritable (Brodie 1989, 1993a,b) and correlated with the
survival of adults (Snell et al. 1988, Jayne and Bennett 1990a), thereby affecting their
fitness. Thus, locomotor performance is subject to natural selection (Garland 1988,
Jayne and Bennett 1990a,b). If the physiological performance of *T. hammondii* is compromised due to *X. laevis* toxins, the risk of predation to the snake might increase because of the decreased physiological performance. Compromised performance might also result in a decreased efficiency of finding additional food items (Huey et al. 1984, Brodie and Brodie 1990).

The physiological performance (maximum speed, sprint speed, endurance, and acceleration) of lower vertebrates has been examined in relation to such factors as temperature (Bennett 1980, Putnam and Bennett 1981, Hertz et al. 1982), state of hydration (Pough et al. 1983, Gatten 1987), gut content (Garland and Arnold 1983, Huey et al. 1984), age (Huey et al. 1990), reproductive condition (Seigel et al. 1987, Jayne and Bennett 1990b, Shine 2003), and a response to toxic prey (Brodie and Brodie 1990). Most studies examining the locomotor speed of squamate reptiles have focused on the maximum speed (Garland and Losos 1994). Finkler and Claussen (1999) suggested that this measure might not provide the most effective illustration of actual performance – maximal locomotion is infrequent during the “normal activity” of squamates (Hertz et al. 1988), and thus not as important to the species as mean speed and/or endurance.

In this study, I examined the effect of consumption of *X. laevis* on the locomotor ability of *T. hammondii*. I measured physiological performance as both sprint speed and endurance of the snakes (Plummer 1997). In addition to examining these measures after snakes were fed *X. laevis*, I compared the obtained values to those recorded when the snake had an empty gut and after ingesting a similar mass of *Lepomis* spp., a nontoxic prey item.
MATERIALS AND METHODS

Individuals of *T. hammondii* were collected in San Diego County, California on 5 June 2001 and 31 May 2002. Eleven *T. hammondii*, 5 males and 6 females, were used in this study. All but one individual were collected from upland populations, naïve to *X. laevis*. Snakes were housed in standard Neodosha™ fiberglass cages (30x30x60 cm) lined with newspapers. Water was supplied *ad libitum*. Before feeding trials began or after they had concluded, snakes were maintained on a diet of appropriately-sized frog (*Rana* sp.) larvae or sunfish (*Lepomis* sp.), offered once or twice per week to the snake in the water dish in the cage. Snakes were kept at a relatively constant temperature (22-25 °C) at all times during housing and trials.

Individuals of *X. laevis* were collected in San Diego and Orange Counties, California, in June 2003 or purchased from Xenopus 1, Inc. (Dexter, Michigan). Similar sized *X. laevis* were housed together in aquaria or 380 l cattle drums. Water was changed as needed and all frogs were fed a commercially-available pellet food at least once weekly.

All trials were conducted on a 2-m long, 9-cm wide racetrack. The floor was lined with Astroturf (Brodie and Brodie 1990, Jayne and Bennett 1990b). Walls of the track (approximately 15 cm high) were painted with a semi-gloss paint so that snakes could not climb the sides. The racetrack was kept in the same room that housed the snakes. This arrangement served to reduce the amount of stress on the animal that would be experienced by relocating it for trials.
Immediately prior to initiating a trial, an individual snake was removed from its cage, and its mass (± 0.1 g) was recorded using an Ohaus® triple beam balance. For each trial, a snake crawled down the racetrack four separate times. Each replicate was separated by 3 min. The time from start to finish (± 0.01 sec.) was measured for each replicate using a digital stopwatch. I calculated the mean speed of the four replicates to define sprint speed (m/sec). The difference between the speed of the last replicate and the speed of the first replicate was the calculated measure of endurance (S.J. Downes, pers. comm.). Thus, the larger endurance value is indicative of a lower amount of fatigue. Snakes were stimulated to crawl by repeatedly tapping the floor of the racetrack directly behind them, and on the tail if necessary (Brodie and Brodie 1990). Every effort was made to ensure that a snake would attempt to flee rather than attack or recoil the head. If the snake refused to crawl in a replicate, however, the trial was discarded (Plummer 1997), and attempted at a later date.

**Physiological/locomotor performance**

To control for differences in locomotor capacity as a function of the presence of prey items in the gut, five trials were conducted in which snakes had not been fed for at least the previous 48 h. These empty stomach trials were conducted twice every week before feeding trials began. No trials were conducted when snakes were in periods of ecdysis (de Queiroz 2003). Empty stomach trials allowed for the comparison of performance between snakes with an empty gut and individuals recently fed. Empty stomach trials were conducted from 7 August to 4 November 2003.
Feeding trials were conducted from 26 August 2003 to 9 March 2004. A snake was offered one or more *Lepomis* or *Xenopus*, depending upon the trial type being conducted, in order to achieve the desired prey mass. Relative prey mass was kept relatively constant (8-10 % of the mass of the snake) across all trials. I implemented a relative prey mass of 8-10 % because it was between the 5 % relative prey mass used by Barthalmus and Zielinski (1988) and the 15-20 % relative prey mass used by Zielinski and Barthalmus (1989). Keeping the prey mass relatively constant among all trials eliminated the bias in locomotor performance resulting from a varied amount of gut content (presence of food has been documented as affecting locomotor performance in snakes; Garland and Arnold 1983, Huey et al. 1984, Ford and Shuttlesworth 1986).

Feeding trials were conducted no more than once per week for each snake. Thirty min elapsed after consumption of the prey item before the trial was initiated (Brodie and Brodie 1990). If a prey item was accepted, this feeding event replaced the normal diet, and the snake was not offered another food item until the following trial. Because some snakes refused to eat *Lepomis* or *Xenopus* during a scheduled trial, alternate prey items were offered for food (e.g., *Rana* larvae). Five *Lepomis* trials and five *Xenopus* trials were attempted for each subject. Because of possible long-term impacts of *Xenopus* toxins affecting future trials, *Lepomis* trials were conducted prior to *Xenopus* trials for each individual. Snout-vent lengths, SVL (± 0.1 cm), were recorded for each individual upon completion of all trials.
Statistical Analyses

Sprint speed and endurance were regressed on the body mass and SVL measurements. Some of the snakes either did not accept *Lepomis* or did not crawl down the racetrack following the consumption of *Lepomis* for all five *Lepomis* trials. Thus, an unequal number of trials among treatment types existed. A total of 55 (11 individuals at 5 trials each) empty stomach trials, 50 *Lepomis* trials, and 55 *Xenopus* trials were conducted. Therefore, the mean sprint speed and endurance of an individual’s five trials were calculated for each treatment type (i.e., the mean of the means). The use of means, rather than the use of an individual’s entire dataset (i.e., all five trials within a treatment), in statistical analyses served to create equal sample sizes across all treatment types. In order to test for a compromised physiological performance of *T. hammondii* due to *Xenopus* toxins, the mean sprint speeds and endurance values were compared among all three trial types using single factor analyses of variance (ANOVA) with mean body mass of individuals as a covariate to control for any effect caused by body size. Statistical analyses were performed using SPSS (SPSS Inc., 2003). Statistical significance was accepted at $\alpha < 0.05$ for all tests.
RESULTS

Morphometrics

Neither mass nor SVL differed with respect to sex of the snakes (p = 0.052 and p = 0.080, respectively). The mass of *T. hammondii* ranged from 27.4-207.5 g (mean ± 1 standard error [SE] = 51.1 ± 15.7 g). Because the mass of an individual was recorded each time that a trial was conducted, these values are based on the mass recorded on the date of an individual’s first trial. In order to keep prey items at a relatively equal mass across all snake individuals, a wide size range of *X. laevis* (1.88-21.60 g, mean = 4.27 ± 0.67 g) was used. These values were taken from the mass recorded throughout the series of *X. laevis* trials, and indicate the entire amount of frogs fed to an individual snake (i.e., the mass is not necessarily reflecting a single *X. laevis* because often times more than one frog was necessary to reach the desired prey mass of 8-10 % of the body mass of the snake).

Physiological performance

There was no correlation between sprint speed and body mass (p = 0.29) or SVL (p = 0.33) of the individual. Similarly, there was no correlation between endurance and body mass (p = 0.45) or SVL (p = 0.34). An analysis of the first five trials for an effect of experience on trial performance indicated that subjects did not increase their speed with repeated runs down the racetrack (F_{4,49} = 0.343, p = 0.85).

Regardless of the trial type, a wide range of sprint speed values was recorded among individuals of *T. hammondii* (Table 1). Similarly, a wide range of endurance values existed among the subjects (Table 2).
Sprint speeds did not differ among treatment types ($F_{2,30} = 2.18, p = 0.13$; Fig. 1). Adding subject body mass as a covariate did not affect the outcome of the analysis ($F_{2,29} = 2.26, p = 0.12$). Endurance of individuals did not differ among treatment types ($F_{2,30} = 1.55, p = 0.23$; Fig. 2). Adding subject body mass as a covariate did not alter the outcome of the analysis ($F_{2,29} = 1.55, p = 0.23$). Mean speed decreased among the four replicates within trials for empty stomach ($t = -5.00, p < 0.001$), *Lepomis* ($t = -3.56, p < 0.001$), and *Xenopus* ($t = -3.71, p < 0.001$) treatments (Fig. 3). The y-intercepts of the regression lines representing each treatment level differed from zero for snakes with empty stomachs ($t = 19.91, p < 0.001$), fed *Lepomis* ($t = 16.85, p < 0.001$), and fed *Xenopus* ($t = 16.54, p < 0.001$).


DISCUSSION

Physiological trials

Effect of snake size

Contrary to Jayne and Bennett (1990b), neither locomotor performance measure (sprint speed or endurance) was dependent on the size (mass or SVL) of the subjects in this study. Although Jayne and Bennett (1990b) used a different species (*T. sirtalis fitchi*), because of relatedness, it is unlikely that one species within the genus would exhibit a locomotor performance dependent upon size when another would not.

Jayne and Bennett (1990b) had a much larger sample size (*N* = 497) and included neonatal individuals, which were documented to have both lower burst speeds and endurance measures than larger individuals. Approximately 95 % of the subjects used by Jayne and Bennett (1990b) were smaller in SVL than the mean value of the individuals of this study (454 mm). The disparity of the range of snake body sizes used between this study and Jayne and Bennett (1990b) is the likely cause for differing conclusions. Had I included neonatal individuals, a difference of performance due to body size might have been detected. The relationship of body size and locomotor performance is something that could be examined in future studies investigating locomotor performance of *T. hammondii*.

Empty stomach vs. recently fed

The additional mass of ingested prey item(s) might impede locomotion and alter the behavior of predators (Shine 1980). Previous studies have documented the
effect of gut content on locomotor performance (Garland and Arnold 1983, Huey et al. 1984, Ford and Shuttlesworth 1986). Usually, the greater the amount of gut content the more lethargic the subject. Typical meals for snakes of the family Colubridae (that family including *Thamnophis*) range from 15-30 % of the body mass of the snake (Fitch 1965). Both Garland and Arnold (1983) and Huey et al. (1984) documented a decreased endurance of individuals fed relative prey masses of 17.6-26.9 % and 14-32 % (mean = 23 %), respectively. Prey items in this study had relative masses well below these values, however, and the presence of food did not compromise the speed or endurance of two-striped garter snakes. Presumably, had a larger relative mass of prey items been implemented, the effect of gut content might have been apparent.

Influence of *X. laevis* toxins/future work

*Xenopus laevis* secrete toxins eliciting oral dyskinesia in certain snake species (Barthalmus and Zielinski 1988, Zielinski and Barthalmus 1989). This oral dyskineasia prevents the snake from consuming the toxic prey item. Within the genus *Thamnophis*, many species include toxic amphibians in their diet (Carpenter 1952, Brodie and Brodie 1990, Brodie et al. 1991, McCallum 1994), and *T. hammondii* consumes *X. laevis* with no sign of oral dyskinesia (Ervin and Fisher 2001). Results of the present study indicate that *X. laevis* toxins also have no effect on the locomotor performance of two-striped garter snakes. Snakes did not increase their sprint speed with increased trial experience, meaning that a temporal bias in physiological performance resulting from the order of treatment presentation should not be considered. Because *Xenopus* trials were conducted last, this result indicates that
increased experience on the racetrack could not be masking the effects of *Xenopus* toxins.

The oral dyskinesia observed in snakes fed *X. laevis* (Barthalmus and Zielinski 1988, Zielinski and Barthalmus 1989) results from the absorption of the neuroleptic-mimicking toxins across the oral mucosa. Because the yawning/gaping is attributed to an oral reaction to/absorption of the toxins, it is unlikely that the resistance of *T. hammondii* to toxins is due to a neutralization of the toxins within the gut of the snake.

Individuals with a greater locomotor performance have an increased fitness due to longer lives and more mating opportunities, and thus, physiological performance is subject to natural selection (Garland 1988, Jayne and Bennett 1990a,b). A compromised physiological performance is associated with an increased likelihood of mortality due to greater predation risks (due to an inability to escape) and/or a decreased efficiency of finding prey (Brodie and Brodie 1990). Because the physiological performance of *T. hammondii* is not negatively affected after predation of *X. laevis*, it would appear that there are no increased risks to snakes associated with consuming this toxic frog. Thus, natural selection should not act against the predation of *X. laevis* by *T. hammondii*. While negatively impacting the majority of the native aquatic wildlife in California, established populations of *X. laevis* provide *T. hammondii* with an additional food source.

All subjects in this study readily fed on *X. laevis* with no observable effects on their physiological performance. Ten of the eleven subjects that willingly accepted *X. laevis* were collected from locations lacking introduced populations of *X. laevis*. Individuals of *T. s. sirtalis* collected from regions where populations of *X. laevis* do
not exist also exhibit a lack of observable behavioral effects (Foster and Mullin, unpubl. data). Because two-striped garter snakes, naïve to *X. laevis*, were able to consume this toxic species without any negative effects I suggest that an innate resistance of *X. laevis* toxins exists in this snake species. The results of this study further document *T. hammondii* as a generalist predator (Rossman et al. 1996). Future studies should focus on the response to *X. laevis* toxins by other species of *Thamnophis* sympatric with the introduced populations of *X. laevis* (e.g., *T. sirtalis fitchii* and *T. sirtalis concinnus*). Such studies would allow comparisons of the resistance to the toxic skin secretions of *X. laevis* among *Thamnophis* species.

Several species of the genus *Thamnophis*, including *T. hammondii*, feed readily upon anuran species within the genus *Bufo* (Arnold 1978, Heinen 1995), which are also toxic anurans. The bufonids contain toxins similar to those within the *X. laevis* skin secretions – both genera contain bufotenidine and serotonin (Daly et al. 1987). This overlap of toxins between the two genera might explain the tolerance that *T. hammondii* has to *X. laevis* skin secretions. If *T. hammondii* is able to ingest *X. laevis* because of similarities between its integumentary toxins and those of *Bufo* spp., other snake species able to consume *Bufo* might be able to eat clawed frogs as well (e.g., other species of the genus *Thamnophis*, *Heterodon* spp., and *Agkistrodon* spp.; Conant and Collins 1991).

Testing snake responses to the individual chemical compounds that make up the suite of toxins of *X. laevis* skin secretions would provide a more definitive answer to why some snakes (e.g., *Thamnophis*) can eat *X. laevis* while other members of the same subfamily (e.g., *Nerodia*; Barthalmus and Zielinski 1988) cannot. Once the
specific toxin or toxins causing the gaping behavior are discovered, a range of doses of the appropriate chemicals could be administered for testing the quantity needed to elicit this gaping behavior observed in *Nerodia*. It would be interesting to know if the same chemicals cause oral dyskinesia in *T. hammondii* at concentrations higher than that naturally occurring in *X. laevis*.

**Conservation/Management Implications**

Despite posing a risk to some native species, there is no doubt that, where populations of the two-striped garter snake and the introduced African clawed frog co-exist in California, *X. laevis* is beneficial to the snake as an additional food source (Mahrdt and Knefler 1972, Ervin and Fisher 2001). Additionally, predatory fish (centrarchids; McCoids and Fritts 1980), the bullfrog (another exotic anuran to California; Mahrdt and Knefler 1972), and herons (Crayon and Hothem 1998) might eat *X. laevis* in California. Thus, *T. hammondii* is one of several predators that might play a role in controlling the population size of the introduced clawed frogs. Field studies could be designed to quantify the dynamics of the Thamnophis-Xenopus relationship (i.e., the effect that one species has on the other). Such studies would be critical in determining the importance of *T. hammondii* in management strategies for reducing populations of *X. laevis*. 
LITERATURE CITED


Table 1 – Minimum, maximum, and mean (± 1 SE) sprint speed (m/sec) of *Thamnophis hammondii* (N = 11) recorded on a 2-m long, 9-cm wide racetrack between 7 August, 2003, and 9 March, 2004, when snakes had an empty gut, and after snakes had recently been fed *Lepomis* spp. or *Xenopus laevis*.

<table>
<thead>
<tr>
<th>Trial type</th>
<th>Minimum sprint speed</th>
<th>Maximum sprint speed</th>
<th>Mean sprint speed (± 1 SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No prey</td>
<td>0.10</td>
<td>0.62</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td><em>Lepomis</em></td>
<td>0.08</td>
<td>0.46</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td><em>Xenopus</em></td>
<td>0.08</td>
<td>0.54</td>
<td>0.25 ± 0.01</td>
</tr>
</tbody>
</table>
Table 2 – Minimum, maximum, and mean (± 1 SE) endurance (the difference between the velocity of the fourth replicate and that of the first replicate within a trial; m/sec) of *Thamnophis hammondii* (N = 11) recorded on a 2-m long, 9-cm wide racetrack between 7 August 2003, and 9 March 2004, when snakes had an empty gut, and after snakes had recently been fed *Lepomis* spp. or *Xenopus laevis*. [The higher endurance values correspond to lower amounts of fatigue to the subject. Because a positive endurance value implies a gain in energy, all values > 0 are reported as 0].

<table>
<thead>
<tr>
<th>Trial type</th>
<th>Minimum endurance</th>
<th>Maximum endurance</th>
<th>Mean endurance (± 1 SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No prey</td>
<td>-0.45</td>
<td>0</td>
<td>-0.11 ± 0.02</td>
</tr>
<tr>
<td><em>Lepomis</em></td>
<td>-0.32</td>
<td>0</td>
<td>-0.07 ± 0.01</td>
</tr>
<tr>
<td><em>Xenopus</em></td>
<td>-0.29</td>
<td>0</td>
<td>-0.08 ± 0.01</td>
</tr>
</tbody>
</table>
Fig. 1 – Mean (+ 1 SE) sprint speed (m/sec) of *Thamnophis hammondii* (N = 11) recorded on a 2-m long, 9-cm wide racetrack between 7 August, 2003, and 9 March, 2004, as a function of gut content (empty, *Lepomis* spp., or *Xenopus laevis*).
Fig. 2 – Mean (± 1 SE) endurance (the difference between the velocity of the fourth replicate and that of the first replicate within a trial; m/sec) of *Thamnophis hammondii* (N = 11) recorded on a 2-m long, 9-cm wide racetrack between 7 August 2003, and 9 March 2004, as a function of gut content (empty, *Lepomis* spp., or *Xenopus laevis*). [The higher endurance values correspond to lower amounts of fatigue to the subject].

**Gut Content**
Fig. 3 – Mean (± 1 SE) sprint speed (m/sec) of *Thamnophis hammondii* (N = 11) of each replicate within all trials for each treatment type (empty stomach, *Lepomis* spp., or *Xenopus laevis*) as recorded on a 2-m long, 9-cm wide racetrack between 7 August, 2003, and 9 March, 2004.
HABITAT USE OF CENTRAL RATSNAKES (Elaphe spiloides) IN AN AGRICULTURALLY-FRAGMENTED FOREST ENVIRONMENT

ABSTRACT

Habitat fragmentation often results from anthropogenic activities such as agriculture, urban development, and resource extraction, and produces an increased amount of edge habitat. The central ratsnake (Elaphe spiloides) is a large, semi-arboreal snake. In this study, I investigated the habitat use patterns, tested for a preference of edge habitat, and determined the thermoregulatory effectiveness of central ratsnakes in an agriculturally-fragmented forest landscape in Clark County, Illinois. Using radio-telemetry, three ratsnakes were relocated every 1-2 days. The subjects appeared to prefer forest and forest edge habitats to agricultural fields and grasslands. In contrast to the findings of studies in the northeastern U.S. and Ontario, however, ratsnakes did not appear to prefer edges to forest interior habitat. Ambient temperatures and snake body temperatures were higher in forest edges than in forest habitat. The thermoregulatory effectiveness of snakes did not differ between forest and edge habitats, possibly accounting for the lack of edge preference by these snakes.
INTRODUCTION

Habitat fragmentation often results from human activities such as agricultural practice, urbanization, and the extraction of resources (Cooper and Walters 2002), and is one of the largest threats to biodiversity (Wilson 1992, Debinski and Holt 2000). The impact of human-caused fragmentation is particularly severe in the midwestern U.S. (Nupp and Swihart 1998). The negative effects of fragmentation include a reduced size of suitable habitat (Donovan et al. 1995), an increased distance between suitable habitat patches (Andren 1994), and an increased exposure to parasites and predators (Robinson et al. 1995). Fragmentation also creates an increased proportion of edge habitat, or ecotone, within a landscape (Anderson and Burgin 2002).

Edges are important features of a landscape (Leopold 1936, Harris 1988, Murcia 1995) and are recognized as functional components of the environment (Wiens et al. 1985, Gosz 1991, Forman 1995). An increased amount of edge habitat (typically examined in association with forested habitat; Keller and Heske 2000, Anderson and Burgin 2002, Blouin-Demers and Weatherhead 2002a) is frequently accompanied by a variety of negative impacts. An increased amount of edge subjects an area to more extreme abiotic influences such as variable wind and temperature patterns (Saunders et al. 1991). Edges are also linked to an increase in predation and/or competition among species (Bolger et al. 1991, Donovan et al. 1995, McCollin 1998) and with the range expansion of exotic species (Yahner 1988, Saunders et al. 1991, Murcia 1995).

Most studies examining the negative impacts of edges have focused on forest-bird species (Gates and Gysel 1978, Wilcove 1985, Chalfoun et al. 2002). Observed
effects of edges on forest-bird species typically include a decline in nesting success in this habitat type (but see Tewksbury et al. 1998). Other species might benefit from an increased amount of edge habitat at the expense of the survivorship of forest-bird species. The decline in nesting success of forest-birds is likely due to certain edge-residing nest predators (e.g., mesopredators – crows [Corvus brachyrhynchos], blue jays [Cyanocitta cristata], raccoon [Procyon lotor], opossum [Didelphis virginiana], and ratsnakes [Elaphe spp.]; Crooks and Soule 1999, Schmidt 2003).

Study species natural history

Ratsnakes are large (up to 175 cm total length, TL), semi-arboreal snakes (Conant and Collins 1991), which prey upon small mammals and birds and their eggs (Fitch 1963, Jackson 1970, Hensley and Smith 1986). The central ratsnake (Elaphe spiloïdes; after Burbrink 2001) ranges between the Appalachian Mountains and the Mississippi River. In Illinois, E. spiloïdes is more common in the southern forests, but occurs statewide in forest, shrub, and edge habitats (Phillips et al. 1999). The central ratsnake mates from April-June, laying between 10 and 20 eggs in May-July. The eggs take about two months to hatch, and hatchlings are 30-35 cm TL (Phillips et al. 1999).

Ratsnakes often hibernate in groups of 10-60 individuals (Blouin-Demers et al. 2000) in communal hibernacula (Lougheed et al. 1999). Individuals exhibit fidelity to overwintering sites, returning to hibernate at the same location from year to year (Prior and Weatherhead 1996, Lougheed et al. 1999, Blouin-Demers et al. 2000). Such a hibernaculum often exists in a rocky area and/or exposed tree roots with numerous fissures and crevices. The hibernaculum is typically located on a sloped terrain.
Home range size has been examined in ratsnakes (Stickel and Cope 1947, Fitch 1963, Durner and Gates 1993, McAllister 1995, Mullin et al. 2000); estimations of home range vary from 1.4 ha (Weatherhead and Hoysak 1989) to 28.3 ha (Stickel et al. 1980) with other studies reporting sizes between these values. Several studies have reported a larger home range size for males than for females (Weatherhead and Hoysak 1989, Mullin et al. 2000). Individual snakes often show philopatry and stay within the same area from year to year (Stickel et al. 1980, Weatherhead and Hoysak 1989, Lougheed et al. 1999).

Ratsnakes exhibit a preference for edges, using this habitat type more often than by chance (Weatherhead and Charland 1985, Durner and Gates 1993, Blouin-Demers and Weatherhead 2002a; but see Keller and Heske 2000). This edge preference by ratsnakes might be due to an increased number of avian (Gates and Gysel 1978, Paton 1994) and small mammalian prey (Weatherhead and Charland 1985, Blouin-Demers and Weatherhead 2001a) in this habitat type. Nest predation is common in edges, and is the leading cause of nest failure for most passerine birds (Ricklefs 1969, Wehtje 2003). Miniaturized video cameras placed at nests have documented that ratsnakes are an important, and in some cases dominant, component of nest-predator assemblages (Thompson et al. 1999, Weatherhead and Blouin-Demers 2004).

Edge preference by ratsnakes might also reflect their increased ability to thermoregulate in this habitat due to increased sun exposure (Weatherhead and Charland 1985). Blouin-Demers and Weatherhead (2001b) reported that thermoregulation after ingestion of large meals is advantageous to snakes because it facilitates quicker digestion. Quicker digestion time is critical as the ingestion of large
meals impairs locomotion, increasing susceptibility of the snake to predators and prohibiting other activities (e.g., searching for a mate or another meal; Greenwald and Kanter 1979). A retarded digestion might also be potentially dangerous, even fatal, to the snake as slowed digestion can allow the decomposition of the prey item inside the gut of the snake (Nalleau 1983). Ratsnakes are more likely to move following feeding events, suggesting that they do increase thermoregulation to facilitate digestion (Blouin-Demers and Weatherhead 2001b,c). Additionally, gravid females utilize edge habitat more often than nongravid females and males (Blouin-Demers and Weatherhead 2001b), indicating that gravid females seek warmer temperatures to decrease gestation time. Elevating body temperatures is also critical during ecdysis (Gibson et al. 1989, Peterson et al. 1993). For these reasons, thermoregulation might be the most important factor in Elaphe habitat use patterns (Grant 1990, Reinert 1993, Blouin-Demers and Weatherhead 2001b).

Objectives

The majority of Illinois is cropland (70 %; Bretthauer and Edgington 2003), fragmenting and reducing much of the original Illinois forests. Only 30.4 % of the pre-settlement forests in Illinois remain (Bretthauer and Edgington 2003). Croplands are the majority of available habitat in Clark County, Illinois (62.3 %; IDNR 2003), where this study was completed. In addition to a loss of habitat, agricultural practices are responsible for creating an increased amount of edge habitat. The great amount of forest edges evident at the Clark County, Illinois, study site might create a favorable environment for central ratsnakes.
One objective of this study was to determine the habitat use patterns of central ratsnakes in a heavily fragmented forest in Clark County, Illinois. Additionally, the use of forest edges by ratsnakes was examined. If edges are preferred due to increased thermoregulatory abilities, then ratsnakes should have body temperatures closer to their preferred body temperature range while in edges than while in other habitat types (Blouin-Demers and Weatherhead 2002a). I calculated and compared the thermoregulatory effectiveness of ratsnakes occupying forest and forest edges. Additional goals of this study included the description of the location, position, and structure of the associated hibernacula and the calculation of the home ranges of individuals during the activity season.
MATERIALS AND METHODS

Study site

The study site, approximately 280 ha in size, was located on privately-owned land in Clark County, Illinois (Fig. 1). Agriculture row-crops (soybeans \textit{[Glycine max]} and corn \textit{[Zea mays]}) surrounded and fragmented a mixed mesic hardwood forest in a ridge-valley system, creating a large amount of edge habitat. Dominant woody vegetation in the forests included beech \textit{(Fagus)}, oak \textit{(Quercus)}, and maple \textit{(Acer)} trees mixed sparsely with pines \textit{(Pinus)} in certain areas. Prevalent understory growth in the forest was made up of an assortment of ferns and mosses, mayapple \textit{(Podophyllum)}, Virginia creeper \textit{(Parthenocissus)}, stinging nettles \textit{(Urtica)}, and poison ivy \textit{(Toxicodendron)}. The ecotone between the forest and the agricultural fields was dominated by species found in the forest understory, with an increase in blackberry \textit{(Rhubus)}, multiflora rose \textit{(Rosa)}, and pokeweed \textit{(Phytolacca americana)}.

Rocky hillsides with potential hibernacula sites were located in the forest habitat. Various creeks or “hollows” have eroded the topsoil, leaving sandstone outcroppings. These creeks flow into a relatively young (approximately 12 years old) man-made lake of approximately 30 ha (created by the structure 9-Mill Creek reservoir), which has fragmented the forest habitat. The surrounding row-crop agriculture has also fragmented and reduced the forest habitat. County Road 1400 E bisected the site, and a power line fragmented the forest habitat in the western area of the site (Fig. 1).
Sampling and data collection

Property owners in the study area granted permission to search their land for snakes (R. Szafoni, Illinois Department of Natural Resources [IDNR], pers. comm.). Based on topographic maps and previous visits to the area, suspected den sites were surveyed intensively beginning in late March and early April 2003 during emergence from hibernacula. Additional areas, especially edge habitats, were searched intensively (rolling logs, rocks, etc.) as the season progressed and temperatures warmed.

Mullin et al. (2000) cautioned against the acceptance of the reported large home ranges using mark-recapture studies (Fitch 1963, Stickel et al. 1980). Mark-recapture studies allow the identification of many demographic parameters (Fitch 1985), but radio-telemetry allows for a more accurate description of habitat use by allowing the researcher to consistently relocate individual animals (Ramsey and Usner 2003). For this reason, I employed the use of radio-telemetry in this study. Collected individuals were marked by clipping a unique combination of ventral scales (Blanchard and Finster 1933). Additionally, a radio transmitter (Model SI-2T, Holohil Systems, Ltd., Ontario, Canada) was surgically implanted into each snake (see Appendix II for procedure). Subsequent relocations in the field were used to calculate the subject’s home range.

The radio transmitters used in this study weighed 12.95 g (less than 6 % of a subject’s body mass; see Appendix II), had a battery life of 24 months at 30 °C, and included thermistors (calibrated prior to implantation) that provided snake core body temperature based on the rate of emitted pulses. Thus, body temperatures (± 0.1 °C)
could be calculated without disturbance to the snake. These values were compared to ambient temperatures recorded at the time of the relocation approximately 10 cm above the substrate in the shade. Prior to the surgical implantation of transmitters, the subject’s mass (± 0.1 g) and snout-vent length (SVL; ± 0.5 cm) were recorded. The gender of each individual was also determined at that time using a cloacal probe.

All subjects were relocated daily or on alternate days using a Telonics TR-4 receiver (Wildlife Materials, Inc., Carbondale, Illinois). Relocations during ingress and emergence identified the exact location of the utilized hibernacula in the area. The coordinates of each snake location were recorded using a Magelllan Map 410 Global Positioning System (GPS).

Data analysis

All data were tested for normality using Kolmogorov-Smirnov tests. Statistical significance was accepted at $\alpha < 0.05$ for all tests. All statistical analyses on thermoregulation data were calculated using SPSS (SPSS Inc., 2003).

Spatial data

All spatial data were analyzed using ArcView Geographic Information System (GIS v 3.2, ESRI, Redlands, California) with the Xtools Extension (DeLaune 2000), Spatial Analyst Extension, and Animal Movement Extension (Hooge and Eichenlaub 2000). Illinois Digital Orthophoto Quadrangle (DOQ) maps were imported into ArcView GIS, and exact locations of individuals were plotted using UTM coordinates. This allowed for the calculation of home range size of each individual using the minimum convex polygon method (MCP; Jennrich and Turner 1969). Minimum convex polygon estimation was chosen because of its extensive use in other spatial

Habitats were defined and quantified within home ranges by digitizing the DOQ maps using ArcView GIS. I described the habitat use of subjects and assigned forest edge habitat as a separate habitat type in order to determine the degree of its use. Following Blouin-Demers and Weatherhead (2001a,b,c, 2002a,b) an individual snake was defined as using edge habitat when it was within 15 m of either side of the boundary between forest and any open (e.g., agriculture, grasses) habitat type.

Thermoregulation data

Thermoregulation data were analyzed for central ratsnakes occupying only forest and edge habitat because individuals were consistently located within these 2 habitat types. Following Blouin-Demers and Weatherhead (2001b; modified from Hertz et al. 1993):

\[ T_{set} = \text{range of 26.5-29.8 °C} \]  

[Eq. 1]

where \( T_{set} \) is the preferred body temperature range for ratsnakes as determined in a controlled laboratory setting (Blouin-Demers and Weatherhead 2001c).

If \( T_b \) is below the \( T_{set} \), where \( T_b \) is snake body temperature, then

\[ d_b = 26.5 - T_b. \]  

[Eq. 2]

If \( T_b \) is above \( T_{set} \), then

\[ d_b = T_b - 29.8. \]  

[Eq. 3]

If \( T_a \) is below the \( T_{set} \) where \( T_a \) is the ambient temperature, then

\[ d_e = 26.5 - T_a. \]  

[Eq. 4]
If $T_a$ is above $T_{set}$, then

$$d_e = T_a - 29.8.$$  \[Eq. 5\]

Following Blouin-Demers and Weatherhead (2002a):

$$d_e - d_b = \text{effectiveness of thermoregulation}$$ \[Eq. 6\]

and is the difference between the deviations of the ambient temperature from the preferred body temperature range ($d_e$) and the deviations of the body temperature from the preferred body temperature range ($d_b$). I used Eq. 6 to compare the abilities of thermoregulation in forest and forest edges. Negative values of $d_e - d_b$ arise when snakes use thermally favorable habitats less than their availability, zero represents thermoconformity, and positive values indicate snakes that are thermoregulating efficiently. Values of $d_e$ and $d_b$ were not calculated while a snake was located underground during hibernation. Univariate analyses of variance (ANOVA}s) were conducted on all thermoregulation data (non-independence of data was corrected by treating each snake as a block).
RESULTS

Three ratsnakes, all males, were collected during the 2003 activity season. Mean mass (± 1 standard error) for these individuals was 726.7 ± 188.3 g, and mean SVL was 121.5 ± 23.9 cm (Table 1). Snakes were relocated a total of 127 times. Due to the tendency of snakes to remain in the same location for extended periods of time, only 51 sample points (for the determination of home range size and habitat selection) were obtained. The mean calculated home range size was 11.22 ± 7.93 ha (Table 2), and individual home ranges are provided (Fig. 2,3,4).

Spatial data

The areas of available habitat types were not uniformly distributed within the combined home ranges of all individuals (Table 2), and subjects appeared to use the eight habitat types non-randomly. One subject was initially collected on a landowner’s driveway (0.13 % of available habitat) and was observed occupying a building (0.41% of available habitat) adjacent to the driveway only once thereafter. Examining the dominant habitat types only, snakes appeared to prefer forest and forest edges and avoided open habitats such as agricultural fields and grasses altogether. Snakes did not appear to prefer forest edges to forest interiors.

Thermoregulation

Mean ambient temperature at time of subject relocation was 20.43 ± 0.80 °C in forest habitat and 21.68 ± 0.52 °C in forest edge habitat. Mean body temperature was 19.47 ± 0.71 °C in forests and 20.89 ± 0.47 °C in forest edges. Mean thermoregulatory effectiveness values (d_e – d_b) were -0.98 (± 0.43) for forests and
-0.87 (± 0.27) for forest edges. Snake body temperatures were strongly correlated with ambient temperatures for each individual and for data pooled among individuals ($r^2 \geq 0.48, p < 0.001$; Fig. 5).

Ambient temperature differed between forest and forest edges ($F_{1,113} = 35.04, p < 0.001$). Ratsnakes maintained different $T_b$ values between forest habitat and edges ($F_{1,111} = 16.79, p = 0.003$). The percentages of time that $T_b$ values were within $T_{set}$ for forest habitat and edges were 2.7% and 3.0%, respectively. The mean deviations of $T_b$’s from $T_{set}$ differed between forest and edge habitat ($F_{1,97} = 7.50, p = 0.01$). Thermoregulatory effectiveness ($d_e - d_b$), however, did not differ between forest and edge habitat for ratsnakes ($F_{1,97} = 0.821, p = 0.37$). Corrected for body size, thermoregulatory effectiveness did not differ among snakes ($F_{2,97} = 0.563, p = 0.57$).
DISCUSSION

Hibernacula structure/location

Two snakes were followed to their overwintering sites where I located two separate hibernacula. Both hibernacula were located on east-facing embankments. Both structures had small (approximately 1 m wide) creeks located nearby (less than 10 m) on the east side. The creek banks were likely created by erosion chiefly due to the runoff of water and flooding of the associated ravines. Both hibernacula were located on steep slopes (greater than 45°) in deciduous forest habitat. One hibernaculum utilized was located in the fissures and crevices of a sandstone facing beneath exposed tree roots. Another snake overwintered in a hole beneath exposed tree roots located in bare soil, but covered heavily by leaves during the time of its use. No other ratsnakes were observed using either hibernaculum during Autumn 2003, even though this species is known to hibernate communally (Lougheed et al. 1999).

Habitat use

Ratsnakes in this study appeared to use habitats non-randomly. Of the eight defined habitat types, only four were common at this site (Table 2). Snakes appeared to occupy forest and edge habitats more often and avoided open areas such as agricultural fields and grasslands. The avoidance of open habitat types is most likely due to the lack of cover that such habitats provide to the snake. In open habitats, lacking shelter, the snake becomes susceptible to predators. Red-tailed hawks (Buteo jamaicensis), numerous at the study site (pers. obs.), often eat ratsnakes (Fitch 1963, Fitch and Bare 1978). Additionally, forest habitat is suitable for many vertebrate prey
species. The vegetative structure of row-crop agriculture does not support high
densities of small mammal and bird species (Vance 1976), important prey items to
ratsnakes. A high proportion of agricultural fields in the study area (see Fig. 1)
reduces the overall habitat quality for ratsnakes (Durner and Gates 1993).

In this study, ratsnakes appeared to display a preference for forests and forest
edges as compared to open habitat types (agricultural fields and grasses). Despite
using a generous definition of edges (± 15 m, as opposed to ± 10 m employed by
Keller and Heske [2000]), however, ratsnakes do not appear to exhibit a preference for
edges over forest interiors. This is consistent with the findings of Keller and Heske
(2000) who studied a nearby population of ratsnakes at Middle Fork Fish and Wildlife
Area, Vermillion County, Illinois (110 km north of Clark County, Illinois). The
similarity in the findings of these two studies indicates a lack of edge preference by
ratsnakes in this geographic region. Because the amount and types of various habitats
differ geographically, the habitat use of a species can often vary throughout different
parts of its geographic range (Weatherhead and Prior 1992). Further studies should be
conducted on the habitat use patterns of ratsnakes in various regions for comparisons
to be made among populations throughout its range.

A preference by ratsnakes for forest edges over forest interiors was not
apparent, perhaps due to the large number of relocation events recorded late in the
season. These late season relocations do not correspond to bird breeding seasons (a
factor explaining the preference for edges by ratsnakes in other studies; see Gates and
Gysel 1978, Paton 1994). Additional surveys of individuals earlier in the season
would more accurately depict ratsnake habitat use throughout the activity season and
could reveal a preference for edges. Weatherhead and Charland (1985) and Blouin-Demers and Weatherhead (2001a) report a stronger preference of edges late in the season, however. It is interesting to note that the first captured ratsnake of the season (12 May 2003) was captured in edge habitat and contained four *Tamias striatus* in the gut (prey that is potentially abundant in edge habitat at all times of the year; Baack and Switzer 2000).

Because ratsnakes in this study appeared to lack an edge preference, it can be inferred that prey is equally abundant within forest and edge habitat, and/or thermoregulatory effectiveness does not differ between these two habitat types. Other studies have suggested that the preference of edge habitat by ratsnakes exists due, in part, to edges being a thermally superior environment for ratsnakes (Blouin-Demers and Weatherhead 2002a). I determined that edges were not a superior thermal habitat, as the thermoregulatory effectiveness of snakes did not differ between forest and edge habitat (discussed below).

**Home ranges**

Home range sizes of all individuals were between previously reported values of 1.4 ha (Weatherhead and Hoysak 1989) and 28.3 ha (Stickel et al. 1980). The home range size of one individual (snake #1), however, was much larger than the observed home range sizes of the other two subjects (752 % larger than the second largest reported home range; Table 1). Much of the habitat within the home range of snake #1 was comprised of agricultural fields (Table 2). This habitat type was never occupied by a subject during the course of relocation events (Table 3). Thus, agriculture is a grossly under utilized habitat type to this species. This habitat type
was centrally located within the home range of snake #1. Active selection of useful habitats surrounding the agriculture by this individual might account for the relatively large home range size (i.e., it had to select areas of useful habitat surrounding the agricultural fields, inflating the size of its home range).

Because the home range size of snake #1 reaches an asymptote after 6 relocation events (i.e., the home range is not increasing in size after additional relocations are added; Fig. 6A), this home range estimation is accurate. The home ranges of the other two subjects increased in size with their last relocation points, and the home range of snake #2 increased in size with each new relocation (Fig. 6B). The disparity in home range sizes between snake #1 and the other two subjects could also be a reflection in the accuracy of home range estimations (Fig. 6), most likely due to the tracking dates of the subjects (Table 1). In Illinois, ratsnakes typically mate from April-June (Phillips et al. 1999), and it is well documented that male ratsnakes exhibit high activity levels while searching for a mate (Gibbons and Semlitsch 1987, Gregory et al. 1987, Durner and Gates 1993). The tracking dates of snake #1 correspond to the ratsnake mating season. I suggest that the larger home range of this individual was also due to its active search for a mate. Had tracking dates occurred during the mating season for all individuals, home range estimations would have been larger and more accurate.

**Thermoregulation**

Ambient temperature differed between forest and forest edges. Forest edges were warmer than forest interiors. Edge habitats possessed relatively open canopies, increasing the sun exposure. An increased amount of sun exposure creates a warmer
environment, and edges have been associated with warmer temperatures in other studies (Flaspohler et al. 2001, Kolbe and Janzen 2002).

Ratsnakes in central Illinois maintained higher body temperatures in edge habitat than in forest habitat due to higher ambient temperatures common to edges. The subjects were not able to maintain a narrow range of body temperatures. Instead body temperatures of the subjects varied greatly and were dependent upon the ambient temperatures (Fig. 5), indicating thermoconformity rather than thermoregulation. Calculated values of Eq. 6 were approximately equal to zero in both forests and edges, also indicating thermoconformity of the subjects. Snakes did not thermoregulate more efficiently in edges, but were able to maintain higher body temperatures in this habitat type primarily because of higher ambient temperatures. After correcting for body size, thermoregulatory effectiveness did not differ among snakes. It is important to correct for body size, because larger snakes have smaller surface area to volume ratios, and thus experience smaller body temperature fluctuations.

Effectiveness of thermoregulation might have been affected by the time of day that relocation events occurred. Out of 127 relocations, 118 were made before 1200 h. During these morning relocations, the snakes might be in the process of thermoregulating, but had not attained optimum temperature. In future studies examining thermoregulation abilities of ratsnakes, it would be advantageous to monitor individual snake body temperatures regularly throughout the day. Although costly, I recommend the implementation of automated radio-telemetry data loggers (see Blouin-Demers and Weatherhead 2001b) for the continuous and more accurate monitoring of snake body temperatures.
Due to differences in altitude, latitude, and climate between the two geographic regions, I hypothesize that differences exist between the preferred body temperature ranges of the Clark County, Illinois, ratsnake population and the Ontario and New York populations (see Blouin-Demers and Weatherhead 2001c). Differing preferred body temperatures between snakes inhabiting the two geographic regions might cause individuals to select habitats having different ambient temperatures, and would account for the different habitat use patterns exhibited by snakes in each region. If they exist, differing preferred body temperature ranges between the two regions could also explain the similarity in this study between ratsnake body temperature and ambient temperature recorded in each of the two microhabitats. I suggest that future work examine the preferred body temperatures of ratsnake populations occupying different geographic regions.

Some limitations of this study should be acknowledged. A small sample size of three individuals also creates an increased uncertainty in the accuracy of mean home range and body sizes. The total number of relocations (127) and sample points (51) are based upon only one field season’s worth of data collection and three subjects. Thus, thermoregulation data is non-independent, although I did account for this non-independence of data by treating each snake as a block (previously described in the materials and methods). Calculated home range sizes were between previously reported values of 1.4 ha (Weatherhead and Hoysak 1989) to 28.3 ha (Stickel et al. 1980), but the home ranges of 2 subjects were considered inaccurate (Fig. 6). Because of the size restriction created by the mass of the radio transmitters, only individuals with a mass of 215 g or greater were able to be radio-telemetered. Weatherhead and
Charland (1985) suggest that individuals of extreme sizes (e.g., very young, small snakes) might exhibit different habitat use patterns than the average sized individuals. Therefore, habitat use patterns described by this study can only be related to ratsnakes of a similar size to the subjects used in this study.

Snakes remained in the same location for longer than one day on most occasions. For this reason, future studies addressing both habitat use and thermoregulatory abilities could be accomplished with fewer relocations per week. In the absence of automated radio-telemetry data loggers (see Blouin-Demers and Weatherhead 2001b,c), snakes should be relocated several times throughout a single day in order to record changes in body temperature with the fluctuating ambient temperatures. Rather than locating an individual once every day, it might be advantageous for studies addressing thermoregulation to re-locate the individual at different times during one day, but for only two days during a given week. This would reflect a more accurate depiction of the thermoregulation abilities in ratsnakes without a loss in accuracy of habitat use.
LITERATURE CITED


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Leopold, A. 1936. Game Management. Scribners, New York, New York, USA.


Table 1 – Morphometrics (snout-vent length [SVL] and mass) and inclusive tracking dates for three male central ratsnakes (*Elaphe spiloides*) radio-tracked in Clark County, Illinois, during the 2003 activity season.

<table>
<thead>
<tr>
<th>Snake #</th>
<th>SVL (cm)</th>
<th>Mass (g)</th>
<th>Tracking Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>130.8</td>
<td>910</td>
<td>19 May-15 July</td>
</tr>
<tr>
<td>2</td>
<td>76.2</td>
<td>350</td>
<td>29 July-12 November</td>
</tr>
<tr>
<td>3</td>
<td>157.5</td>
<td>920</td>
<td>29 July-12 November</td>
</tr>
</tbody>
</table>
Table 2 – Area (ha) of available habitat types within each individual home range for three male central ratsnakes (*Elaphe spiloides*) radio-tracked in Clark County, Illinois, during the 2003 activity season.

<table>
<thead>
<tr>
<th>Habitat types</th>
<th>Snake 1</th>
<th>Snake 2</th>
<th>Snake 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural Field</td>
<td>12.00 (44.31 %)</td>
<td>–</td>
<td>0.25 (6.93 %)</td>
</tr>
<tr>
<td>Buildings</td>
<td>–</td>
<td>–</td>
<td>0.01 (0.41 %)</td>
</tr>
<tr>
<td>Deciduous Forest</td>
<td>8.39 (30.99 %)</td>
<td>1.71 (57.62 %)</td>
<td>1.18 (32.70 %)</td>
</tr>
<tr>
<td>Edge Habitat</td>
<td>6.17 (22.80 %)</td>
<td>1.24 (41.71 %)</td>
<td>1.84 (50.97 %)</td>
</tr>
<tr>
<td>Grasses</td>
<td>0.38 (1.41 %)</td>
<td>0.02 (0.67 %)</td>
<td>0.29 (8.03 %)</td>
</tr>
<tr>
<td>Residential Yard</td>
<td>–</td>
<td>–</td>
<td>0.03 (0.82 %)</td>
</tr>
<tr>
<td>Road/Driveway</td>
<td>0.12 (0.47 %)</td>
<td>–</td>
<td>0.00 (0.13 %)</td>
</tr>
<tr>
<td>Water (Pond/Lake)</td>
<td>0.01 (0.03 %)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total Area (home range)</td>
<td>27.08</td>
<td>2.97</td>
<td>3.60</td>
</tr>
</tbody>
</table>
Table 3 – Number of occasions that three male central ratsnakes (*Elaphe spiloides*) were located in specific habitat types within Clark County, Illinois, during the 2003 activity season.

<table>
<thead>
<tr>
<th>Habitat types</th>
<th>Snake #1</th>
<th>Snake #2</th>
<th>Snake #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buildings</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Deciduous Forest</td>
<td>7</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Edge Habitat</td>
<td>8</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Road/Driveway</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
Fig. 1 – Digital Orthophoto Quadrangle map of the Rocky Hollow region in Clark County, Illinois, (approximately 280 ha) that was intensively searched for timber rattlesnakes (Crotalus horridus) during the 2003-04 activity seasons.
Fig. 2 – Minimum convex polygon home range estimation of central ratsnake (*Elaphe spiloides*) #1, radio-tracked in Clark County, Illinois, during the 2003 activity season.
Fig. 3 – Minimum convex polygon home range estimation of central ratsnake (*Elaphe spiloides*) #2, radio-tracked in Clark County, Illinois, during the 2003 activity season.
Fig. 4 – Minimum convex polygon home range estimation of central ratsnake (*Elaphe spiloides*) #3, radio-tracked in Clark County, Illinois, during the 2003 activity season.
Fig. 5 – Scatter plot of ambient temperatures and body temperatures (°C) of central ratsnakes (*Elaphe spiloides*) radio-tracked during the 2003 activity season in Clark County, Illinois. A) snake #1, B) snake #2, C) snake #3, and D) pooled data for all three individuals.
Fig. 6 – Minimum convex polygon home range size estimations for each increasingly-larger data set of relocation points (when the subject actually moved) for central ratsnakes (*Elaphe spiloides*) radio-tracked during the 2003 activity season in Clark County, Illinois. A) snake #1, B) snake #2, and C) snake #3.
SURGICAL PROCEDURES FOR RADIO TRANSMITTER IMPLANTATION

The following protocol was used for surgical implantation of radio transmitters in central ratsnakes (Elaphe spiloides). The procedure is adopted from the techniques reported in Reinert and Cundall (1982), Reinert (1992), and Hardy and Greene (1999, 2000).

- Transmitter dimensions are 4.8 cm in length and 1.5 cm in width, and each has a 46 cm whip antenna. Each transmitter assembly weighs less than 6% of a subject’s body mass, and is coated with a biologically-compatible plastic.
- Collected subjects (adults only) were fasted for at least 24 hours prior to surgery. Ambient temperature at the time of surgery was 20 °C.
- A subject was restrained on a flat horizontal surface in a clear plastic tube that is slightly larger in diameter than its head. The tube was capped at one end after introducing a gauze pad dampened with 1 ml liquid isofluorane anaesthetic. The tube cap had a hole to accept ventilation tubing that assists cardio-respiratory recuperation following surgery. The subject typically entered the surgical plane of anesthesia within 8 to 12 min. Depth of anesthesia was gauged by loss of righting reflex and response to external stimuli (e.g., touch, pinch).
- Once anesthetized, the subject was rolled on its side and the surgical site aseptically prepared with povidone-iodine solution. Sterile surgical instruments were used for all procedures. Proper sterile techniques (including mask and sterile gloves) were utilized.
• A 25-mm longitudinal incision was made with scissors between scale rows 2 and 3, at a point 2/3 the length of the body from the head. The skin was reflected away from ribs and towards the ventral scutes. The peritoneum was identified and incised longitudinally to accommodate the transmitter body in the intraperitoneal space.

• The transmitter was inserted with the antenna protruding cranially from the incision. A 48-cm fine-bore brass canula was tunneled subcutaneously anterior from the incision. The antenna was inserted into the tube and then the tube is withdrawn through a small incision at the anterior end of the tube between scale rows 2 and 3.

• To prevent the transmitter from migrating within the subject’s body cavity, the transmitter was tethered to the medial side of the intracostal muscle mass with 5-0 suture.

• The incision was closed in a single layer (no separate closure for the peritoneum) using an interrupted lateral “mattress stitch” with 5-0 absorbable suture and knots tied on the superior side of the incision (to minimize contact with substrate when crawling). This stitch serves to evert the skin edges so that the dermal layers are in apposition to facilitate rapid healing.

• If the subject was still in the surgical plane of anesthesia as the incision closure is begun, the cranial end of the restraining tube was opened and the gauze removed so that the subject began its recovery phase in anticipation of the procedure’s completion.
• Following skin closure, a volume of sterile saline solution equivalent to 4% of the subject’s body mass was injected intraperitoneally using a 23-gauge needle at a point 15 cm anterior to the vent. This fluid, to prevent dehydration, replaced any that was lost during the procedure or due to interstitial and intracellular edema.

• As this procedure involves minimal tissue trauma, analgesic was not employed. Furthermore, antibiotics were not administered because they might eliminate the animal’s normal internal fauna.

Post-operative Care

• The subject was monitored until adequately recovered from anesthesia (judged by return of righting reflex, voluntary movement and appropriate response to stimuli). At this point, the tube was withdrawn and the subject was returned to a holding cage maintained at 25 °C and monitored frequently to ensure an uneventful recovery.

• 48 hours following the procedure, the snake was offered a small food item (e.g., 30 g mouse). The snake was maintained in the holding cage until it accepted the food item and defecated, or until one week had passed since the procedure. Extended post-operative stays were avoided because of the additional stress placed on the subject.

• Subjects were released at site of capture.