Nest-site selection in Eastern hognose snakes (*Heterodon platirhinos*)

by

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Thesis submitted to the Department of Biology in partial fulfillment of

the

requirements for the B.Sc. Honours degree, specialization Biology

University of Ottawa

Ottawa, Ontario

May 3, 2010

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Supervisor's signature

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Abstract

Nest-site selection is considered to be the only form of parental care in most oviparous reptiles, as they abandon their eggs after laying them. Previous studies have shown that egg incubation conditions affect offspring phenotype. Thus, female reptiles can increase offspring fitness by selecting nest-sites which have optimal conditions. I examined nest-site selection in Eastern hognose snakes, *Heterodon platirhinos*, which excavate their own nests. I compared nest-sites to randomly selected sites that were not used by snakes. Nest-sites had higher mean temperatures than random sites and their temperatures were closer to the known optimal incubation temperatures of other temperate Colubrid snakes. Temperature variance was higher in nest-sites than in random sites. I also quantified nest-site microhabitat, and found that ground cover vegetation differed significantly between nest and random sites. These results show that Eastern hognose snakes select nest-sites that offer developmental temperatures that are likely to maximize offspring fitness and identify habitat characteristics that may be involved in the selection of nest-sites. I suggest that further nest-site research be conducted on Eastern hognose snakes due to their status as a Threatened species in Canada.

Introduction

Most oviparous reptiles abandon their eggs after laying them. This means that the only form of parental care is the selection of the site in which the eggs will develop. Nest-site selection has thus been studied extensively in reptiles (Garrick and Lang, 1977; Burger and Zappalorti, 1986; Doody et al., 2006; Escalona et al., 2009; Pike et al., 2010). The physical conditions an egg experiences during incubation affect offspring phenotype and survival (Shine, 2003; Brown and Shine, 2004). One of the most important factors that affects offspring development during incubation is temperature (Booth, 2006). Incubation temperatures can determine neonate sex, and influence offspring morphology and behaviour (Shine, 2003; Booth, 2006). Experimental studies on the effects of incubation temperatures on snake neonate development have found evidence that mean incubation temperature, and temperature variance, have implications for offspring fitness (Burger, 1989; Burger, 1990; Burger, 1998; Blouin-Demers et al., 2004; Patterson and Blouin-Demers, 2008). These findings show that female snakes can potentially increase the fitness of their offspring by selecting nest-sites with optimal incubation temperatures (Blouin-Demers et al., 2004; Booth, 2006).

The habitat characteristics of a nest-site are also important because they determine the temperature within it, and may influence whether a female chooses to use a site (Burger and Zappalorti, 1986). Although descriptions of snake nest-sites exist for a number of species (Sexton and Claypool, 1978; James and Henderson, 2004; Cunnington and Cebek, 2005), few studies actually quantify nest-site microhabitat selection (Burger and Zappalorti, 1986). By comparing nest-sites to available but unused surrounding sites, the habitat characteristics that are important in nest-site selection can be identified (Burger and Zappalorti, 1986).

In this study I compared the temperature profiles and habitat characteristics of nest-sites excavated by Eastern hognose snakes, *Heterodon platirhinos*, with randomly selected sites. This allowed me to determine whether female snakes select sites that maximize offspring fitness, and which habitat characteristics are important in nest-site selection.

Eastern hognose snakes are heavy-bodied snakes that are thought to reach maturity at four to five years old (Seburn, 2009). Female snakes lay 25 eggs on average, with clutch size being dependant on body size (Seburn, 2009). Eastern hognose snakes were an ideal species for this study due to their specific nesting behaviour. Female snakes excavate sandy soil to create a burrow in which to lay their eggs (Cunnington and Cebek, 2005). Because the snakes excavate the nests themselves, rather than locating nest-sites opportunistically, they are a good choice for a study on nest-site selection (Burger and Zappalorti, 1986). A pile of sand at the opening of the nest also makes nest-sites easy to identify (Cunnington and Cebek, 2005).

Temperature Profiles

I hypothesized that Eastern hognose snakes select sites that maximize offspring fitness. To test this I compared nest-site temperatures to the temperatures of randomly selected sites. Although no incubation studies have been conducted on Eastern hognose snakes, several studies of other temperate Colubrid snakes have found optimal incubation temperatures ranging from 28 to 30 °C (Burger, 1989; Burger, 1990; Blouin-Demers et al., 2004; Patterson and Blouin-Demers, 2008). Eggs incubated at these temperatures resulted in offspring with maximal fitness when compared to offspring incubated at other lower and higher temperatures (Burger, 1989; Burger, 1990; Blouin-Demers et al., 2004; Patterson and Blouin-Demers, 2008). Evidence has also been found which suggests that offspring fitness is higher when there is low variance in incubation temperatures (Patterson and Blouin-Demers, 2008). Because the Canadian populations of Eastern hognose snakes are at the northern limit of their range, female snakes will most likely select sites that have the highest possible temperatures throughout the incubation period (Shine, 2004). If nest-sites are selected to maximize offspring fitness they should therefore: 1) have temperatures that are closer to known optimal incubation temperatures (when compared to random sites); 2) have lower variance in temperature than random sites; 3) have a higher mean temperature over the incubation period than randomly selected sites.

Microhabitat Selection

The habitat characterisation portion of my study was based on a previous study on nest-site selection conducted by Burger and Zappalorti (1986). Although this study examined nest-site selection in pine snakes, *Pituophis m. melanoleucus*, it is similar because both snake species excavate nests in sandy soil and exhibit the same digging behaviour (Burger and Zappalorti, 1986; Cunnington and Cebek, 2005). Burger and Zappalorti (1986) found that nests had more grass than random sites, and that herbs and shrubs were more likely to be found on random sites as opposed to nest-sites. Nests also had significantly less canopy cover and were further from edges than random sites (Burger and Zappalorti, 1986). I expected that the similar nesting behaviour of Eastern hognose snakes would produce the same results as Burger and Zappalorti’s study.

Materials and Methods

The study took place on the Manestar tract of St. Williams Crown forest in Norfolk County, Ontario, Canada. All sites were located within an open area of grassy dunes on the southern side of the tract. Nests were located beginning on June 25, 2009, and were identified by the distinctive sand pile at their entrance. Female snakes were observed excavating several of the nests in the same manner as described by Cunnington and Cebek (2005). In addition to this, more than one female was observed in several of the nests. However, because I could not be sure which nests were used by only a single female, the nests were not categorized as communal and solitary nests, but were all treated equally.

Once a nest was located it was marked with flagging tape and a GPS point was recorded. To ensure that nests contained eggs and were not test holes (Cunnington and Cebek, 2005), I excavated each nest until eggs were located. Nine nests were used in the study. Although I discovered more than nine nests, some nests were not usable because no eggs were found within them. The nest depths ranged from 10.4 to 20.1 cm, and each consisted of a long, partially sand-filled tunnel ending in an egg chamber. The tunnels were up to 1 m long.

A random site was then selected for each nest-site. Random sites were chosen by standing at the nest and walking 50 metres in a random direction selected by a compass (see Blouin-Demers and Weatherhead, 2001). All random sites fell within the open dune habitat that is used for nesting by hognose snakes (although one was within a small copse of trees in the middle of the dunes). Once a random site was selected it was also marked and a GPS point was taken.

Temperature Profiles

Temperature logging iButtons (DS1921 and DS1922, Dallas Semiconductor, Sunnyvale, CA) were placed in the nests with the eggs and the nests were then refilled. The eggs were not disturbed or removed. Temperature loggers were coated in silicon to prevent water damage and were attached to flagging tape to aid in recovery at the end of the incubation period.

A temperature logger was placed within random sites at the same depth as their corresponding nest-site. The temperature loggers were programmed to record the temperature every 30 minutes (with the exception of nest-site 9 and random sites 8 and 9 which had temperatures recorded every 60 minutes). The temperature loggers were removed from the sites on September 11, 2009. Data from July 7, 2009 to September 11, 2009 were used in the analysis.

Microhabitat Selection

Several habitat characteristics were measured at the sites. Grass was defined as any grass or grass-like sedge species. Shrubs were defined as perennial species with woody stems, while herbs were defined as annual plant species. Ground cover was measured using a plastic sighting tube with cross hairs at one end (based on Winkworth and Goodall, 1962). The tube was randomly pointed at the ground 20 times within a one metre radius area that was centred on the nest, and the type of vegetative ground cover that intersected the cross hairs was recorded. The results were then multiplied by five to get a percentage. Canopy cover was recorded in the same way. The observer lay next to the site and pointed the tube randomly within a 45 degree cone above them. Only canopy cover from trees with a diameter at breast height larger than 7.5 cm was recorded. Edge was deemed to be the beginning of a continuous stretch of forest. Habitat characterisations were performed before sites were excavated to ensure that the habitat was undisturbed.

Statistical Analyses

Due to the small sample size (N=9 nests), paired *t*-tests were used to analyze the data. In addition to testing for differences between habitat variables at nest and at random sites, I also compared the temperature means and the thermal variation (coefficient of variation) between nest and random sites. An optimal incubation temperature, 28.7 °C, was calculated by averaging known optimal values for other Colubrid snake species (Burger, 1989; Burger, 1990; Blouin-Demers et al., 2004; Patterson and Blouin-Demers, 2008). The deviation of nest-site and random site temperatures from this optimal temperature were compared. All *t*-tests were one-tailed due to my habitat predictions, and my prediction that nest temperatures would be higher and less variable than random site temperatures. Statistical analysis was done using JMP 8.0 (SAS Institute, Inc., Cary, NC) and levels of significance were set to $\alpha = 0.05$. Means ± S.E. are reported.

Results

Temperature Profiles

Nest-site temperatures were closer to the optimal incubation temperature, 28.7 °C, than random site temperatures (**Figure 1**). The difference was significant with a mean deviation of 4.89 ± 0.092 °C for the nest-sites and 6.97 ± 0.541 °C for the random sites ($t_{(8)} = 3.919$, $P = 0.0022$). The mean temperature of the nest-sites was 23.81 ± 0.092 °C, which was significantly higher than the random site mean of 21.73 ± 0.541 °C ($t_{(8)} = 3.919$, $P = 0.0022$). The temperature variance was not significantly higher for the random sites as predicted, but was instead significantly higher for the nest-sites (nest-sites, 0.15 ± 0.005; random sites, 0.12 ± 0.008; $t_{(8)} = 3.429$, $P = 0.0045$). A linear regression of temperature variance (coefficient of variation) on mean temperature revealed a positive relationship, with sites with higher mean temperatures having significantly higher temperature variance ($F_{(1)} = 26.649$, $R^2 = 0.625$, $P < 0.0001$).

Microhabitat Selection

There was significantly more grass present at nest-sites, with a mean of 54.0 ± 4.3%, than at random sites which had a mean of 21.6 ± 7.6% grass ($t_{(8)} = 4.186$, $P = 0.0015$; **Figure 2**). Nest sites averaged 1.1 ± 0.9% herbs and 2.4 ± 2.4% shrubs which was significantly lower than the average values of 12.7 ± 4.2% herbs and 17.3 ± 6.1% shrubs at the random sites (herbs, $t_{(8)} = 2.986$, $P = 0.0087$; shrubs, $t_{(8)} = 2.026$, $P = 0.0387$; **Figure 2**).

There was no significant difference between the canopy cover of nest and random sites with an average of 0.7 ± 0.7% cover over nests and 13.8 ± 8.4% cover over random sites ($t_{(8)} = 1.560$, $P = 0.0787$). There was also no significant difference in the distance to the nearest edge ($t_{(8)}$ = 0.563, P = 0.7056). Nests were on average 14.5 ± 1.4 m away from an edge while random sites had a mean distance of 16.5 ± 3.0 m from the closest edge.

Figures

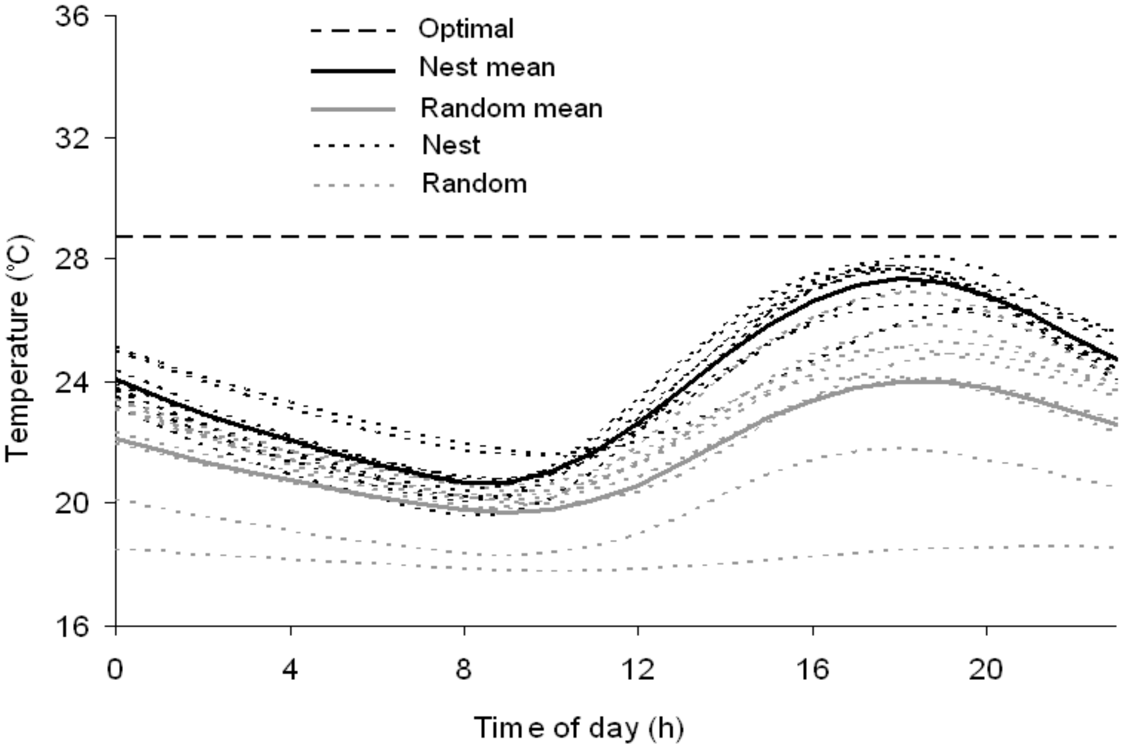

Figure 1. Mean daily temperatures over the 66 day study period, for nest and random sites. Individual site temperatures are given as well as the average daily temperatures for the combined nest-sites and random sites. The optimal temperature is derived from previous studies on Colubrid snakes and is 28.7 °C.

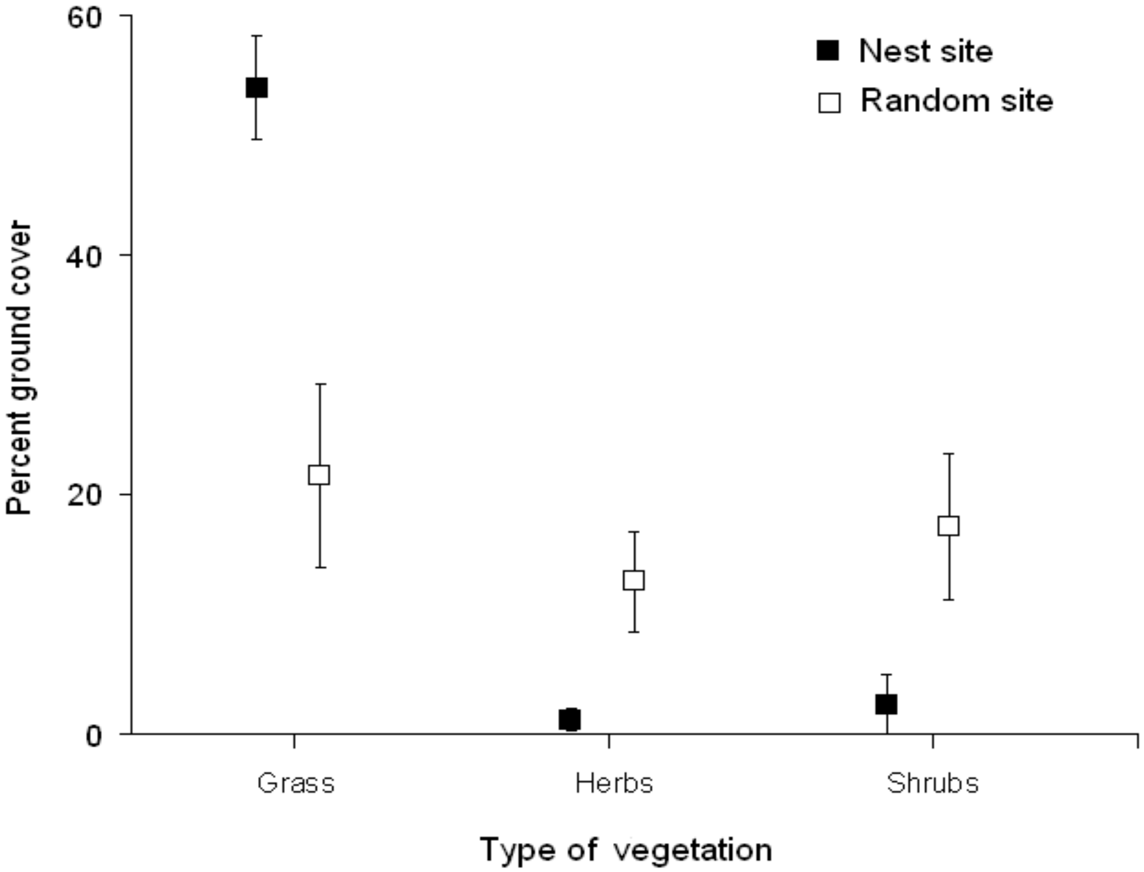

Figure 2. Mean ground cover percentages (± S.E.) at nest and random sites. Differences in means between nest and random sites are significant.

Discussion

Temperature Profiles

Female Eastern hognose snakes selected nest-sites with higher mean temperatures that were closer to the optimal development temperatures of other temperate Colubrid snakes. Although no experimental studies have documented the effects of temperature on this species, I believe that the close similarity in the optimal temperatures of other temperate Colubrid snakes indicates that there is little variation between species (Burger, 1989; Burger, 1990; Blouin-Demers et al., 2004; Patterson and Blouin-Demers, 2008). Therefore, this study provides evidence that Eastern hognose snakes select nest-sites with developmental temperatures that likely maximize offspring fitness.

Although the nest-site mean temperature, 23.8 ± 0.1 °C, was higher than the random site mean, 21.7 ± 0.5 °C, it was still much lower than the optimal mean (28.7°C). This could be attributed to the northern location of this population's nesting habitat, but a study on black rat snake, *Elaphe obsoleta*, nests at the same latitude recorded higher temperatures (Blouin-Demers et al., 2004). Black rat snake nest mean temperatures ranged from 22.2 to 31.8°C with a communal nest mean temperature of 27.93 ± 0.81 °C (Blouin-Demers et al., 2004). The lower temperatures of Eastern hognose snake nests are therefore likely due to their location within sandy soil as opposed to organic debris. Black rat snakes and other snake species lay eggs in leaves, wood, and other organic litter that releases heat as it decomposes, warming the eggs (Burger and Zappalorti, 1986; Blouin-Demers et al., 2004). Eggs laid in sand only gain heat from solar radiation warming the ground, and so nests are cooler. Shallow nests would be warmer for this reason, but may come with added risks such as increased predation and the risk of egg exposure by weather. Deeper nests may also have a more stable environment with less thermal and hydric variation.

Temperature variance was found to be significantly higher at nest-sites than at random sites which was the opposite of my prediction. Although existing research shows that increased variance decreases fitness in snakes (Patterson and Blouin-Demers, 2008) other studies have found no real effect of temperature variation on fitness (Webb et al., 2001). The correlation between mean temperature and variance also indicates that there may be a trade-off where the benefits of a higher average temperature outweigh the costs of higher temperature variance. Due to the low environmental temperatures available in this nesting habitat (when compared to other snake species), there may be strong selective pressure to choose nests with the highest possible temperatures.

Microhabitat Selection

Ground cover vegetation differed significantly between the nest and random sites and followed my predictions based on nest-site selection in pine snakes. I believe that this is due to the similarity in nesting behaviour between Eastern hognose snakes and pine snakes (Burger and Zappalorti, 1986; Cunnington and Cebek, 2005). Nest-sites had significantly more grass and less herbs and shrubs than random sites. Sites with more herbs and shrubs likely have more root mass beneath the ground and therefore may be more difficult to excavate (Burger and Zappalorti, 1986). A lot of ground cover could also reflect and absorb sunlight, and so reduce the amount of radiant energy reaching the nest itself. Moisture levels have also been shown to affect neonate fitness (Brown and Shine, 2004), so it is possible that increased vegetation has an effect on the amount of water able to reach the eggs. Grass may be more common at nest-sites simply because it is found where shrubs and herbs are not. Sites with grass could also be chosen because they provide some root mass which helps prevent nests from collapsing during excavation (Burger and Zappalorti, 1986).

Although canopy cover and the distance to the nearest edge did not differ significantly between nest and random sites, this is probably due to the study location. Because all nests were located within the same open dune macrohabitat, I selected random sites that were also located within this macrohabitat. This allowed me to identify important variables on a microhabitat scale but decreased the differences between nest and random sites. Comparing nest-sites to random sites located within a different macrohabitat type would likely have led to greater differences, but would have been a pointless exercise since the snakes all nested within the same open dune macrohabitat.

Canopy cover was shown to be significant in Burger and Zappalorti's pine snake study (1986), as sites with less canopy cover will receive more sunlight resulting in higher temperatures. The low percentage of canopy cover over hognose nest-sites, 0.7 ± 0.7%, reveals that canopy cover is an important variable in Eastern hognose snake nesting, with snakes nesting in open areas. The insignificant difference between the amount of canopy cover over nest-sites versus random sites is therefore due to the fact that the random sites are located within the same open macrohabitat.

The distance to the nearest edge probably did not differ significantly for the same reason; however, it is also possible that this variable is unimportant in nest-site selection in this species. Because similar habitat characteristics were found to be significant in both Eastern hognose and pine snakes, these findings may also apply to other burrowing snake species.

Conclusions

A limiting feature of this study was the small number of nests examined. However, despite the small scope of this study, it is the first of its kind on this species and will hopefully lead to further research on nest-site selection in Eastern hognose snakes. It also adds to the body of evidence that female snakes actively select sites that maximize offspring fitness. Eastern hognose snakes are listed as a Threatened species in Canada and so it is important to understand habitat use and selection in this species, as well as incubation conditions.

Acknowledgements

I would like to thank my supervisor, Gabriel Blouin-Demers, for all of his help with this project. In addition, I would like to acknowledge Laura Robson's assistance in characterising habitat and finding nests. Funding for this study was provided by NSERC, WWF, PC and CWF. Finally, I would like to thank the Eastern hognose snakes who provided nests for this study.

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