CHEMOSENSORY AND BEHAVIORAL ECOLOGY OF THE DIPSADID SNAKES: *CONTIA TENUIS*, *DIADOPHIS PUNCTATUS*, AND *HYPSIGLENA CHLOROPHAEA*

By

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To the faculty of Washington State University:

The members of the committee appointed to examine the dissertation of ROBERT ELBERT WEAVER find it satisfactory and recommend that it be accepted

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CHEMOSENSORY AND BEHAVIORAL ECOLOGY OF THE DIPSADID SNAKES: *CONTIA TENUIS*, *DIADOPHIS PUNCTATUS*, AND *HYPISGLENA CHLOROPHAEA*

Abstract

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While much is known about the behavior of many groups of squamate reptiles, including several medium to large bodied snakes (e.g. natricines and viperids), we know very little about many small, cryptic species of snakes. Because of this bias in our current knowledge of the behavior of snakes my dissertation is focused on the chemosensory and behavioral ecology of three species of small-bodied and cryptic snakes, the Sharp-tailed Snake (*Contia tenuis*), Ring-necked Snake (*Diadophis punctatus*), and the Desert Nightsnake (*Hypsiglena chlorophaea*).

My dissertation had three main objectives: 1) To examine the effects of shelter-site and prey odor availability on behavior, 2) To test for prey chemical discrimination among these species, 3) to examine abitoic factors that influence activity patterns. Chapter 1 focused on the effects of shelter availability and prey odor on *H. chlorophaea*. Individual nightsnakes made nocturnal movements and chose shelters in combination with lizard odor, and avoided mouse odor. Chapter 2 described the effects of three moonlight intensities: 1) a new moon, 2) half-moon, and 3) a full moon on the activity

patterns of *H. chlorophaea*. These data show a full moon to have a statistically significant effect on the movement patterns of *H. chlorophaea*. In Chapter 3, I show that *H. chlorophaea* is able to discriminate between two size classes of potential prey. Individuals has a lower latency (time to first tongue flick) and showed a greater mean rate of tongue flick towards a small sized (and ingestible) prey item over a larger, un-ingestible prey item. Chapter 4 shows that *H. chlorophaea* of two size classes (adults and juveniles) do not prefer invertebrate prey, an often repeated statement in both peer-reviewed papers and regional field guides. In Chapter 5, I show that *Contia tenuis* shows a preference for slugs as prey, reaffirming previous, yet unsubstantiated claims by several authors. Chapter 6, shows that shelter and prey odor has an effect on behavior, in the case on a diurnal species, *Diadophis punctatus*. Similar to *H. chlorophaea*, individual *D. punctatus* chose shelters in proximity to a suitable prey odor, in this case a snake, and avoided mouse odors.

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Dedication

My dissertation is dedicated to my star, Kendra Sue.

Without whom none of this would have been possible.

I owe it all to her.

INTRODUCTION

Squamate reptiles (snakes, lizards, and amphisbaenians) are a diverse and species-rich clade of vertebrate with some 8000 described species (Zug et al., 2001). Within squamates, the sub-order serpentes (ca. 2900 species) are equally diverse in terms of biology. Snakes are cosmopolitan in distribution, from the tropics to the Arctic Circle, inhabiting oceans, hot and cold deserts, forest and woodlands to tundra, and range in size from the diminutive threadsnakes (Leptotyphlopidae) to the much larger boas and pythons (Boidae). Because of this broad distribution and wide range in morphology, the ecology and behavior of snakes defies being classified into a single category. Snakes can be diurnal, nocturnal (or both), ambush or wide foraging predators, constrictors, venomous, or may rely on neither method, employing a spectrum of such prey capture strategies (Greene, 1997).

With regards to the phylogenetic relationships of snakes, most research has been conducted on the caenophidia (advanced snakes), which has led to a more complete understanding of the evolutionary history of these snakes (Kelly et al., 2009; Lawson et al., 2005; Wüster et al., 2008). While, we may have a grasp on the relationships of advanced snakes, detailed behavioral studies on many species are lacking. What we do know is biased toward medium to large bodied species. This is due in part to the secretive nature of most snakes, and especially smaller, nocturnal species.

In terms of behaviors related to the chemosensory biology of snakes much data exists on groups such as natricines (Burghardt 1992; 1993; Krause and Burghardt 2001; Luiselli et al., 2007; Savitsky and Burghardt 2000), elapids (Aubret et al., 2004), colubrids (Mori 1993; 1994; Halstead et al., 2008), and viperids (Greenbuam 2004; Clark

2004; Eskew et al., 2009), the end result being an overall incomplete knowledge of such behaviors in other snakes. Because of these biases, my dissertation focuses on the biology of three small, secretive temperate dipsadid snakes, the Sharp-Tailed Snake (*Contia tenuis*), the Ring-necked Snake (*Diadophis punctatus*), and the Desert Nightsnake (*Hypsiglena chlorophaea*).

Contia tenuis is found only on the west coast of the United States, and into southwestern British Columbia (St John, 2002). This species is often encountered in coniferous forests, and oak woodlands, assumed to feed on gastropods, we know very little about the ecology of this species. *Diadophis punctatus* is a trans-continental species, broadly distributed from the eastern and mid-western United States, with a more spotty range in the western United States (Ernst and Ernst, 2003). This species is considered an ecological generalist, and despite being common in some parts of the Pacific Northwest (St John, 2002), its behavior is largely unknown.

The majority of my dissertation is focused on the behavior of *H. chlorophaea*, a species found from portions of northern Arizona, southeastern California north into south-central British Columbia (Mulcahy, 2008), and within this distribution it is most often associated with desert landscapes. *Hypsiglena chlorophaea* feeds largely on lizards (Rodriguez-Robles et al., 1999), but also feeds on a wider range of vertebrate prey (Weaver, 2010). It is nocturnal, and not easily found, thus much of its behavior is subject to speculation. All of these species are of great conservation concern. Both *H. chlorophaea* and *C. tenuis* endangered in Canada, and considered species of concern elsewhere. These data gathered in this dissertation will allow management personal to make informed decisions with regards to policies that affect these species.

Overview of Chapters

Chapter one: *Microhabitat and prey odor selection in Hypsiglena chlorophaea*

In this chapter we studied the effects of various shelter and prey odor combinations on selection of microhabitat characters by the Desert Nightsnake, (*Hypsiglena chlorophaea*), a dipsadine snake, by examining the activity patterns of these snakes over a 23 h period. Three prey odors: lizard, snake, mouse (plus water as control). In the first experiment, each odor was tested separately in various shelter and odor combinations. Our results showed that snakes preferred shelter to no shelter quadrants, and most often selected a quadrant if it also had prey odor in the form of lizard or snake scent. However, snakes avoided all quadrants containing mouse (adult) odor. In the second experiment, all three odors plus water were presented simultaneously. We found that snakes showed a preference for lizard odor over the others, but again showed an aversion to mouse odor, even compared to water. The circadian rhythms in both experiments showed generally the same pattern, namely an initial peak in activity, falling off as they entered shelters, but then again increasing even more prominently from lights off until about midnight. Thereafter, activity tapered off so that several hours before lights on in the morning snakes had generally taken up residence in a shelter. Prey preference correlates with field studies of dietary frequency of lizards, while activity exhibits strong endogenous nocturnal movement patterns.

Chapter two: Effects of simulated moonlight on activity patterns of a temperate dipsadine snake, the desert nightsnake (Hypsiglena chlorophaea)

This chapter addresses the effects of simulated moonlight on 20 desert nightsnakes (*Hypsiglena chlorophaea*) collected from May–August 2008 at a site in central Washington State, USA. Snakes were maintained in captivity using standard husbandry practices. Based upon moon light levels gathered at the collection site, snakes were tested over a 23 hour period under three moonlight trials, new moon (0.05 lux), half moon (0.32 lux), and full moon (2.10 lux). Simulated moon-up during the half moon and full trials was from 2300–0300 hour. I detected no significant difference in the number of movements during either the new or half moon trials. However, snakes made significantly fewer movements from 2300–0300 hour (moon-up) during the full moon trials. For nocturnal species such as *H. chlorophaea* lower activity levels in response to a full moon may effect foraging time and patterns, mate searching behaviors, as well as movements to and from hibernacula. Alternatively, by decreasing activity during periods of bright moonlight, snakes may reduce the risk of predation.

Chapter three: Odor cues allow the desert nightsnake, Hypsiglena chlorophaea (Colubridae: Dipsadinae) to assess prey size

Chapter three looks at prey chemical discrimination in desert nightsnakes (*Hypsiglena chlorophaea*), specifically we sought to see if *H. chlorophaea* can chemically discriminate between two prey size classes (small and large). Twenty-one

adult individuals of *H. chlorophaea* (mean snout-vent length = 364 ± SD) were collected in 2008 from a site in Washington State, U.S.A. We obtained odors assays from a known prey item, the western terrestrial gartersnake (*Thamnophis elegans*) collected at the same site as *H. chlorophaea*. The size classes were a small *T. elegans* (164 mm snout-vent length, SVL) and a large *T. elegans* (640 SVL). We presented all odors on 15-cm cotton swabs held 2.5 cm in front of snake's snout. For each trial we recorded the number of tongue flicks in 60 seconds, and the latency to first tongue flick. We then compared individual snake responses to each prey size class, as well as to odor controls (water and cologne). Our analysis showed no statistically significant difference in latency times when comparing cologne to water, or small snake odor to these controls. In terms of tongue flicks, snakes responded the strongest to the small snake odor. Our study is the first to show that a species of snake can chemically discriminate between sizes of prey.

Chapter four: Prey chemical discrimination by the Desert Nightsnake (Hypsiglena chlorophaea): a comparison of invertebrate and vertebrate prey odor ues

Chapter four is an investigation into the responses of adult and juvenile Desert Nightsnakes (*Hypsiglena chlorophaea*) to possible invertebrate and vertebrate prey. Snakes were collected during 2008 from three localities in Washington State. We obtained odors assays from three possible invertebrate prey: spider (*Tegenaria* spp.), scorpion (*Paruroctonus borealis*), and field cricket (*Gryllus* spp.), and compared responses to those toward a known vertebrate item (*Thamnophis* spp.). All prey items were collected at the same site as *H. chlorophaea*. Odors were presented on 15-cm cotton

swabs held 2.5 cm in front of snake's snout, and we recorded the number of tongue flicks in 60 seconds, and the latency to first tongue flick. We observed no significant difference in tongue flicks or latency between spider, scorpion, or cricket odors. Both adult and juvenile *H. chlorophaea* responded with a higher tongue flick rate to snake odor. Our study shows that *H. chlorophaea* does not exhibit a favorable chemosensory response toward the invertebrates species tested, a result which is supported by current field work.

Chapter five: Behavioral responses to potential prey through chemoreception by the sharp-tailed snake (Contia tenuis)

The Sharp-tailed Snake (*Contia tenuis*) is a small (usually <30 cm total length), cryptic species found along the west coast of the United States and north into southwestern British Columbia. Because of its secretive nature, little is known about its behavioral ecology. In this chapter, we tested behavioral responses of 13 adult *C. tenuis* collected from a site in eastern Washington to potential invertebrate prey odors. We presented snakes with 2 control odors (water, cologne) and 2 possible invertebrate prey odors (earthworm, slug). Overall, there was a significant difference in both the time-to-first-tongue flick (latency) and mean tongue flick rate (number of tongue flicks/60 s trial) for the odors tested. The mean latency period was 6.0 ± 1.87 s for earthworm and 4.1 ± 1.57 s for slug. The mean tongue flick rate for earthworm and slug was 13.8 ± 4.09 flicks/s and 39.7 ± 15.79 flicks/s, respectively. These results support prior claims of a preference for slugs by *C. tenuis*. This preference for slugs may also explain the presence of *C. tenuis* in areas of anthropogenic disturbances with an abundance of slugs.

Chapter six: Effects of shelter and prey odor availability on the behavior of Diadophis punctatus

Chapter six examined the effects shelter and prey odor on the behavior of the Ring-necked snake (*Diadophis punctatus*) over a 23 h period. The prey odors tested were: lizard, snake, mouse (plus water as control). In experiment one each odor was tested separately in various shelter and odor combinations. Results showed that snakes preferred shelter to no shelter quadrants, often selecting a quadrant if it also had prey odor in the form of a snake scent, followed by lizard. However, snakes avoided quadrants containing mouse (adult) odor. In experiment two all three odors plus water were presented simultaneously. We found that snakes showed a preference for snake odor over the others, and showed an aversion to mouse odor. Activity in both experiments showed a similar pattern, namely activity beginning with lights on, peaking mid-day, thereafter, activity tapered off as snakes began taken up residence in a shelter just before lights off. Prey preference correlates with field studies of a diet comprised mostly of snakes (and some lizards) while activity exhibits strong endogenous diurnal movements.

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CHAPTER FORMAT AND ATTRIBUTION

My dissertation is comprised of six chapters, each of which is a manuscript intended for publication. Because of this, each chapter is formatted for the journal in which they are published, or submitted to. In terms of study design, data collection, and the writing and revising of the manuscripts I am the main contributor for each. However, in some cases, my committee chair Kenneth V. Kardong is co-author. Chapter one has been published in the journal *Copeia* and thus formatted accordingly. Chapter two has been submitted to the *Journal of Ethology*. Chapter's three and four are submitted to journals *Behavioral Ecology* and *Journal of Herpetology*. Chapter five is in press in the journal *Northwestern Naturalist*. Chapter six is formatted for the journal *Herpetologica*.

CHAPTER ONE

MICROHABITAT AND PREY ODOR SELECTION IN *HYPSIGLENA CHLOROPHAEA*

Robert E. Weaver and Kenneth V. Kardong

RH: *Hypsiglena* microhabitat and prey choice

Key words: Foraging behavior, nocturnal, activity patterns, Nightsnake

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We studied the effects of various shelter and prey odor combinations on selection of microhabitat characters by the Desert Nightsnake, (*Hypsiglena chlorophaea*), a dipsadine snake. We also examined the activity patterns of these snakes over a 23 h period. Three prey odors were tested, based on field work documenting natural prey in its diet: lizard, snake, mouse (plus water as control). In the first experiment, each odor was tested separately in various shelter and odor combinations. We found that snakes preferred shelter to no shelter quadrants, and most often selected a quadrant if it also had prey odor in the form of lizard or snake scent. However, snakes avoided all quadrants containing mouse (adult) odor. In the second experiment, all three odors plus water were presented simultaneously. We found that snakes showed a preference for lizard odor over the others, but again showed an aversion to mouse odor, even compared to water. The circadian rhythms in both experiments showed generally the same pattern, namely an initial peak in activity, falling off as they entered shelters, but then again increasing even more prominently from lights off until about midnight. Thereafter, activity tapered off so that several hours before lights on in the morning snakes had generally taken up residence in a shelter. Prey preference correlates with field studies of dietary frequency of lizards, while activity exhibits strong endogenous nocturnal movement patterns.

Several factors may influence habitat preference and circadian patterns of activity. Among squamates, microhabitat (e.g., shelter-sites) use varies across size and age class (Langkilde and Shine, 2004; Webb and Whiting, 2006). It may also change within or between seasons (Martin and Lopez, 1998; Beck and Jennings, 2003; Heard et al., 2004), habitats (Beck and Lowe, 1991), or sexes (Brito, 2003; Whitaker and Shine, 2003). Shelters play many important roles, with individuals utilizing sites for thermoregulation (Slip and Shine, 1988), predator avoidance (Downes, 2001; Diaz et al., 2006), or when ambushing prey. This is especially true for some snakes that are often ambush predators (Slip and Shine, 1988; Beck, 1995; Theodoratus and Chizar, 2000; Beverlander et al., 2006).

However, much of the research on shelter selection in squamates has been conducted on primarily diurnal species, such as various species of iguanid (Hertz et al., 1994), agamid (Melville and Schulte II, 2001), or scincid lizards (Klingenbock, 2000; Quirt et al., 2006). Such species use visual cues typically not available to nocturnal species (Heatwole, 1977). What is known about shelter use by small, nocturnal squamates is limited to studies on gekkonid lizards (Kearney and Predavec, 2000; Kearney, 2002) or Australian elapids (Schlesinger and Shine, 1994; Webb and Shine, 1997; Webb and Shine, 1998; Downes, 1999; Webb and Whiting, 2006).

In terms of their behavior, dipsadinae snakes are some of the least known of snakes. This is despite being a very species-rich group, found throughout the Western Hemisphere (Zug et al., 2001). While most species of dipsadine snakes are confined to the Neotropics of Central and South America, some species have distributions that extend into Mexico and north into the United States and southern Canada.

One nearctic species of dipsadine snake is the Desert Nightsnake (*Hypsiglena chlorophaea*). *Hypsiglena chlorophaea* is a small (usually < 60 cm TL), secretive, nocturnal, and little studied snake found from the desert southwest, throughout the intermountain western United States, and north into the Okanagan Valley of south-central British Columbia (Mulcahy, 2008). Throughout its range, *H. chlorophaea* is most often found in dry, rocky habitat (Stebbins, 2003), with an abundance of lizards, on which they commonly feed (Diller and Wallace, 1986; Rodriguez-Robles et al., 1999).

In the Pacific Northwest, *H. chlorophaea* ranges from southern Idaho, into eastern Oregon and Washington (Nussbaum et al., 1983). *Hypsiglena chlorophaea* is a habitat generalist, being found in shrub-steppe dominated by Big Sagebrush (*Artemisia tridentata*), to disturbed range land, and agricultural fields, as well as Oregon White Oak (*Quercus garryana*) savannah, and Douglas-Fir (*Pseudostuga meinziesii*) and Ponderosa Pine (*Pinus ponderosa*) forests (Storm et al., 1995; St. John, 2002; Weaver, 2006).

Hypsiglena chlorophaea is considered a dietary specialist, feeding primarily on sceloporine lizards and squamate eggs. However, the diet in the Pacific Northwest is quite varied. Lizards, *Sceloporus* spp., *Uta stansburiana*, *Plestiodon skiltonianus*, and *Elgaria* spp., juvenile snakes, *Thamnophis* spp., and *Crotalus oreganus*, anurans, *Pseudacris regilla, Anaryxus boreas*, and small mammals (Weaver, unpubl.) have all recorded as prey taken by *H. chlorophaea* of all sizes (Diller and Wallace, 1986; Rodriguez-Robles et al., 1999; Weaver, 2006).

Historically, *H. chlorophaea* has been considered a species of concern in Washington State, and was known from very few specimens (McAllister, 1995). However, recent field work (Weaver, 2006) has shown that *H. chlorophaea* is a somewhat more abundant

snake that can be found in sufficient numbers allowing for specimens to be collected, brought into captivity, and utilized for behavioral studies. Our experiments focused on microhabitat (shelter) selection in *H. chlorophaea* as it relates to the presence or absence of potential prey. To conduct our experiments, we used shelters in combination with three potential prey items (lizard, snake, mouse), plus a control (water). In Experiment one, an individual odor was presented in four combinations with or without shelters. In Experiment two, we presented snakes simultaneously with all three odors, plus the control, and shelters in all. Additionally, we recorded the circadian activity patterns of snakes during both experiments. Our purposes were to identify the effects of shelter and prey odor on microhabitat choice, the relative preference for different prey odors, and the basic circadian activity pattern of *H. chlorophaea*.

MATERIALS AND METHODS

We conducted our experiments with nine adult (five male and four female) *H. chlorophaea* (225–502 mm snout-vent length). All were collected during 2006 from three counties (Kittitas, Klickitat, and Yakima) in central Washington State. Snakes were housed individually in 26 x 51 cm glass aquaria, and maintained on 12:12 light cycle year around (lights on at 8:30 h and off at 20:30 h). Temperatures in both the rooms housing the snakes and where experiments were performed were held at 25–30 C. Snakes were provided with water *ad libitum*, and each snake was alternately fed a variety of prey items

(various species of lizards, snakes, and nestling mice) on an irregular basis. This was done to control for bias that may arise from feeding snakes exclusively one prey species.

Prey items used during the trials included the Western Fence Lizard (*Sceloporus occidentalis*), and Terrestrial Gartersnake (*Thamnophis elegans*), both of which are known prey items of *H. chlorophaea* (Weaver, 2006). Bedding from adult Swiss-Webster mice (*Mus musculus*) was also used as potential mammalian prey. All prey items (except *M. musculus*) were collected from the same localities as *H. chlorophaea*. Snakes were maintained under these conditions for at least six months before experimental trials were begun.

Experiments were conducted using square testing arenas (1.25 m wide x 0.5 m high) constructed out of compressed fiberglass panels, resting on a metal platform 20 cm above the floor. Overhead lighting provided 12 h of simulated daylight, while 20-watt red, incandescent bulbs were used during 12 h of darkness. The floor of the testing arena was covered with plain white butcher paper and divided into four equal quadrants using black tape (Fig. 1). Before each trial a fresh piece of butcher paper covered the arena floor that allowed each marked quadrant to show through. Individual prey odors were presented in covered plastic Petri dishes (diameter = 15 cm), with seven evenly spaced holes (diameter = 1.2 cm) drilled through the top of the dish.

Prey odors were collected by placing one to two specimens each of either a lizard or snake into 400 cc of distilled water (Beverlander et al., 2006). Prey items were swirled gently for about 10 min and then removed. This water was poured into the dish, the bottom of which was lined with filter paper. Soiled bedding from cages containing adult mice was used and enough was added to the dish to cover the bottom (Melchiors and

Leslie, 1985; Lee and Waldman, 2002; Slusarczyk and Rygielesky, 2004; Robert and Thompson, 2007). Controls during each trial consisted of placing a similar amount of distilled water into a dish, again lined with filter paper. During the trials, shelters were provided that consisted of opaque plastic hide-boxes (10 x 6 x 5 cm). Shelters were provided with or without each odor during Experiment one (Fig. 1). During Experiment two, shelters were present with each of the three odors, plus the control. F1

Trials were run for 23 h with one hour for change over (between 17:00 and 18:00 h). Snakes were placed into the center of an arena, and kept under a small plastic cup. This was then lifted at the start of a trial, recording commenced, and all personnel left the room. Behaviors were filmed with Panasonic cameras suspended over each arena and recorded with a Panasonic time-lapse VCR.

Several variables were recorded during playback of tapes. We recorded the amount of time spent in each quadrant in minutes. This was recorded once a snake's head entered a quadrant and until its head left a quadrant. These times were recorded and totaled for each quadrant during each hour.

Experiment one: shelter-site and prey-odor selection.—During this experiment each snake was provided with a combination of a single prey odor (lizard, snake, mouse), and control (demineralized water), with the presence or absence of a shelter. Four combinations were used, one for each of the four quadrants: A: no shelter/prey odor, B: shelter/prey odor, C: no shelter/ no prey odor (water), D: shelter/no prey odor (water) (Fig. 1A). The position of the choices was randomly changed at the beginning of each experimental trial. The order of prey item tested was also randomized for each snake.

Experiment two: prey odor preference.—In this experiment the same three odors were tested simultaneously (lizard, snake, mouse), plus a control (water, Fig. 1B). To control for shelter effects, a hide-box was placed into each of the four quadrants with the door facing the Petri dish holding the odor. Again, similar to Experiment one, the position of the choices was randomly changed at the beginning of each experimental trial, with the order of prey item tested also random.

During both experiments, shelters and Petri dishes were washed between trials with 70% ethanol, rinsed with demineralized water, and allowed to dry overnight. During the set-up of experiments gloved hands (Microflex, non-sterile, latex) were used when handling dishes, shelters, and when changing the paper that covered the bottom of the arena floor. When placing the dishes into the arena we were careful not to cross-contaminate quadrants. One week was allowed to pass between trials of the same snake. Snakes were fed after each trial, confirming hunger.

Statistical analysis.—Each snake was run twice, its score averaged, and these means examined with a non-parametric test (Kruskal-Wallis, *H*-test). When this test produced statistical significance, we performed a Tukey Test (*Q*-score) test of multiple pair-wise comparisons to discover which were significantly different from one another.

RESULTS

Experiment one: shelter-site and prey odor selection.—After placement into the arena at about 18:00 h, Nightsnakes spent the first few minutes in the center of the arena before moving toward the edges. Snakes made several movements around the arena, moving along the walls, and making quick movements across the arena. While making these movements snakes would crawl into and around shelters. Snakes would crawl toward the dishes, usually pausing if a dish contained a prey odor. These behaviors usually lasted for 30 minutes to an hour. All snakes settled into a shelter after one hour and remained in that shelter until lights off. During this time, no part of a snake's body was out of the shelter

Just after lights off (20:30 h), snakes emerged. Often just a head would initially be visible from the shelter opening. After a few minutes snakes would leave the shelter and begin to move around the arena. During these movements snakes would move through quadrants containing shelters, moving into and out of that shelter. Snakes ignored (crawling past, not pausing) dishes that contained no prey odor (water). When a snake crawled near a dish that contained either a lizard or snake odor they would pause while moving their heads from side to side across the top of the dish.

The darkened room did not allow us to confidently count tongue flicks, or record the rate of flicks, but tongue flicks were evident. We observed snakes moving their heads back and forth while making circuitous routes around the dish. This behavior would continue for several hours, until eventually settling into a shelter near a dish usually containing prey odor. Snakes would coil inside the shelter with just their heads visible in the opening of the shelter, pointing toward the dish. They remained in this position for

the rest of the night and into the following day. During trials most snakes behaved in this manner. However, in two trials snakes selected a shelter almost immediately and remained in that shelter for the total duration of the 23 h trial.

During the 23 h trials (54 total) there was a significant quadrant effect for snake (Kruskal-Wallis, H =18.876, $P < 0.001$), lizard ($H = 22.778$, $P < 0.001$), and mouse ($H = 29.098$, $P < 0.001$). During the lizard and snake trials, post-hoc, pair-wise multiple comparisons (Tukey test) revealed a significant preference for quadrants containing a shelter-odor combination (B) over quadrants with odor only (A), or no odor/ no shelter (C). However, there was no preference for quadrant D (no odor/shelter) over quadrant B (shelter/odor; $Q = 2.816$, $P > 0.05$) or A (odor/no shelter; $Q = 2.531$, $P > 0.05$) during the snake or lizard trials (Table 1). T1

During trials when snakes were presented with the mouse odor, most snakes spent significantly less time in a quadrant containing a mouse odor only (A) and significantly more time in a quadrant without mouse odor (C and D). There was, however, no significant difference between quadrant C (no odor/no shelter) or B (odor/shelter, $Q = 0.221$, $P > 0.05$) during the mouse odor trial (Fig. 2). F2

When comparing the presence or absence of a shelter, there was a significant effect of shelter for all trials, snake ($H = 14.899$, $P < 0.001$), lizard ($H = 18.243$, $P < 0.001$), and mouse ($H = 13.704$, $P < 0.001$). This was not true for odor. During both the snake and lizard odor trials there was no difference in selection for quadrants with an odor, or without ($H = 1.766$, $P = 0.184$ and $H = 1.090$, $P = 0.296$ respectively). However, during the mouse trial, there was a significant difference between quadrants with and without odor, the snakes preferring quadrants without mouse odor ($H = 15.393$, $P < 0.001$).

Experiment two: prey odor preference.—As in Experiment one, upon placement into the arena, snakes remained motionless for a few minutes and then moved about the arena, making several circuits, investigating both shelters and dishes. Unlike Experiment one, some snakes continued these movements up to lights out. However, most snakes moved into a shelter and remained there until just after lights out. In only one trial out of 18 did a snake enter a shelter immediately and not emerge for the remainder of the 23 h trial.

When presented with all three odors simultaneously (lizard, snake, mouse) and control (water), each accompanied by a shelter, *H. chlorophaea* showed a preference for the quadrant containing the lizard odor, spending a significant amount of time in that quadrant, over either mouse ($Q = 6.106$, $P < 0.05$), and control ($Q = 3.797$, $P < 0.05$, Fig. 3). Post-hoc comparisons showed no difference between quadrants containing either snake or mouse odor ($Q = 3.322$, $P > 0.05$), and snake or lizard ($Q = 2.784$, $P > 0.05$, Table 2). F3, T2

Experiment one and two: activity patterns.—For each prey type, the trials for *H. chlorophaea* were combined, with the average number of movements for each hour plotted to show activity patterns. Overall, there was no significant difference ($H = 0.2815$, $P = 0.963$) in the average number of movements made during trials for either experiment one or two. Average movements during trials for each prey odor during experiment one were: lizard (mean = 3.25 ± 4.11 SD), snake (3.13 ± 4.43 SD), and mouse (mean = 2.77 ± 4.64 SD). During experiment two when all odors were present, snakes moved an average of 3.44 ± 4.64 SD.

During two trials (lizard and snake), *H. chlorophaea* showed similar bi-modal activity patterns, making several movements during the first few hours, before settling into a shelter before lights out (Fig. 4A and Fig. 4B). Then, after lights out (20:30 h), renewed activity characterized by a steady increase in activity peaking around midnight. Activity continued until 1:00 or 2:00 h, which dropped off thereafter, with only a few individuals making brief movements just before lights on (8:30 h).

After being placed into the arena, snakes were initially more active for the first few hrs (18:00–19:00), making 8.15 and 9.36 moves, respectively (Fig. 4C), during the mouse odor trials. For either the lizard or snake odor trials, snakes made less movements during that two hour span, (4.52 and 4.63 times, and 4.35 and 3.68 times during each hour, Fig. 4A and 4B). Activity decreased just before lights out (20:30) and did not increase again until 22:00 h, about one hour after activity during the lizard or mouse trials, with a peak at 23:00 h. Thereafter, activity levels dropped, with snakes making few movements between 1:00 and 3:00 h. Unlike both the lizard and snake trials, activity during the mouse trials stopped at 6:00 h, with no snakes making any movements just before lights on at 8:30 h (Fig. 4C).

During Experiment two, again we combined both trials of all snakes which were averaged per each hour, and then plotted to show activity patterns. Similar to Experiment one, snakes made several movements during initial introduction. However, some snakes did not settle into a shelter before lights out. Movements plateaued between 19:00 and 21:00 h, with an increase in activity from 22:00 to 23:00 h. Starting at about midnight, activity declined steadily into the morning hours, with all activity stopping at about 6:00 h (Fig. 4D). F4

DISCUSSION

Experiment one: shelter and prey odor selection.—During Experiment one, *H. chlorophaea* (except the two individuals which remained in a shelter the entire time) showed a preference for quadrants with lizard or snake odors that included a shelter over other combinations without a shelter. Time spent in quadrants with such odors and shelter was significantly greater than those with odor alone. With mouse odors, there was a shelter and odor effect, but in a complicated way. Nightsnakes exhibited significantly less interest in a shelter quadrant if mouse odor was present and than if mouse odor was absent (Fig. 2). Some *H. chlorophaea* did initially investigate the quadrant with mouse odor, slowly approaching the dish, but then usually quickly turned away from the dish and moved away in a rapid manner. We interpret these responses to mouse odor, relative to water, as representing a negative preference, even active avoidance of adult mouse odors. Our general observations, reported above, are also consistent with this interpretation.

A strong selection for quadrants with lizard or snake odor (plus shelter) is not surprising. Prior work examining museum specimens (Rodriguez-Robles et al., 1999) and field work in both southwestern Idaho (Diller and Wallace, 1986) and Washington State (Weaver, 2006) revealed *H. chlorophaea* to feed primarily on lizards. However, Weaver (2006) also showed that *H. chlorophaea* take snake prey (*Thamnophis* spp.).

Experiment two: prey odor preference.—Overall, snakes behaved in much the same way during Experiment two (all three prey odors plus control presented simultaneously). Nearly all individuals (83 %) made just a few movements after introduction and then settled into a selected shelter until lights off. Evaluation of choice of snake odor is complicated. There was no significant difference between lizard and snake odor preferences, but there was also no significant difference between snake odor and all other choices either (Table 2). This may reflect natural prey preference or result from the large variation in choices for snake odor in our study. However, a preference for lizard odor quadrants is significant, spending a greater amount of time in those quadrants containing lizard odor (plus shelter), than mouse or control (water). Similar to Experiment one, snakes in Experiment two displayed avoidance behavior when encountering the mouse odor (with or without shelter).

Overall results from both experiments suggest that snakes are not making random movements. The statistical results show a strong selection for the combinations of odors and shelter, especially lizard odor. Little or no time was spent in quadrants lacking a shelter, with or without odor. Snakes avoided quadrants with mouse odor, and qualitative observations indicate such behavior was extreme and may be in response to the odor of an adult mouse as a threat rather than as a food item.

Experiment one and two: activity patterns.—While we observed no significant difference in the activity patterns of *H. chlorophaea* during either experiment one or two, there were distinctive movements and behaviors displayed by *H. chlorophaea* during trials. When first placed into the arena, most snakes moved in a slow irregular manner,

making several movements around the arena. A few snakes made quick, erratic movements, and two snakes moved immediately into a shelter and remained there during the entire 23 h period. In those two trials, the immediate seeking of cover may have been the result of introduction into the arena in spite of our taking great care to introduce the snakes into the arena in a gentle, and stress free manner. In nearly all trials (96%) snakes settled into a shelter after a few minutes of initial orientation within the center of the arena.

During both Experiments one and two, there were two peaks in activity patterns. The first occurred following introduction into the arena, while the second bout of activity started with lights out (20:30 h) and peaked about midnight. Thereafter, snakes tended to settle into a shelter as morning approached and activity waned and all snakes were in a shelter before lights on (8:30 h).

There were only slight differences in activity patterns between the two experimental conditions. During Experiment one activity peaked during 23:00 and 0:00 h, with three snakes making brief movements during the time just before lights on at 8:30 h. Activity levels showed a slow steady decline until 5:00 and 6:00 h. The snakes that made crepuscular movements did so quickly, moving between shelters. During Experiment two, activity peaked an hour earlier at 22:00 h, but again showed a slow steady decline, with all activity ceasing at 6:00 h.

We interpret the first peak in activity related to introduction effects, and the second peak in activity related to intrinsic circadian rhythms. As interpreted by others (Bevelander et al., 2006), we too suggest that the first activity peak may represent investigation of a novel microhabitat and/or be related to the introduction procedure

itself. Other than movements made after introducing an individual snake into the arena, the movements made by *H. chlorophaea* were strictly nocturnal. *Hypsiglena chlorophaea* has been anecdotally reported as being occasionally encountered during the day (Woodbury, 1931; Grimser, 2002), but most encounters in the field are nocturnal. Activity times from the field reported for 74 individual *H. chlorophaea* from May to October ranged from 21:00–0:600 h, with peaks between 23:00 and 1:00 h (Weaver, 2006), very similar to our laboratory activity results reported here. As the common name suggests for this snake, *H. chlorophaea* is nocturnal in habit, sometimes engaged in low levels of crepuscular, pre-dawn movements.

Period of or conditions in captivity could conceivably effect basic prey choice, but this seems unlikely. Pilot studies of snakes collected in the field and run within a few days of capture showed similar shelter-odor choices (Experiment one), odor choices/aversions (Experiment two), and circadian rhythms to snakes in this controlled study. Further, correlation between experimental and field data is also evident in prey preferences. In this study, *H. chlorophaea* showed a statistically significant preference for lizard and snake odors (with shelter) over controls and over mouse odors. These choices are similar to documented prey choices in the field (Weaver, 2006).

While the avoidance of adult mice odor by *H. chlorophaea* is also probably an intrinsic behavior it is interesting to note that using similar protocols, other laboratory studies (Theodoratus and Chiszar, 2000; Bevelander et al., 2006) of shelter-odor choices showed preferences for, not aversion to, mouse odors. The possible reasons for this avoidance by Nightsnakes of adult mouse odors is likely related to its limited defense ability and the resulting vulnerability to rodent retaliation from protective adult mice. In

contrast, the larger (50–60 cm SVL) Western Rattlesnake (*Crotalus oreganus)* feeds on adult rodents and is equipped with the venom apparatus to quickly kill (Kardong, 1986) and the strike and release behavior to protect itself from retaliation (Chiszar et al., 1992). These rattlesnakes show a preference for environmental mouse odors when moving in microhabitats (Theodoratus and Chiszar, 2000). The Pigmy Rattlesnake (*Sistrurus miliarius)* is smaller (38–51 cm SVL), about the same size as large *H. chlorophaea*. But, similar to *C. oreganus*, *S. miliarius* exhibits a preference for mouse odors (and shelter), although the more natural frog prey is slightly preferred (Bevelander et al., 2006). Although small, *S. miliarius* has a venom apparatus capable of injecting a painful defensive bite (Klauber, 1956), and thereby is able to meet a challenge even from an adult mouse. However, *H. chlorophaea* possesses no such specialized venom apparatus to rapidly kill its prey or to effectively inflict immediately painful defensive bites. Nesting adult mice may inflict damage (incisor teeth) while protecting their young. The behavior displayed during the mouse trials indicates that *H. chlorophaea* may avoid large adult mice as they would any other possible threat.

The idea that *H. chlorophaea* is "venomous" is an old idea (Cowles, 1941), often repeated in field guides today. This unqualified claim is unwarranted for several reasons. *Hypsiglena chlorophaea* does not possess a venom gland but instead a Duvernoy's gland (Taub, 1967) associated with a tooth that is neither hollow nor grooved (Young and Kardong, 1996). Although such systems are sometimes termed "venom systems" (Jackson, 2007), this is a premature conclusion until experimental studies verify directly that it is actually deployed in rapid killing of prey and/or in successful defense (Kardong, 1996). The oral glands and associated teeth of *H. chlorophaea* are unlike the hollow

fangs and true venom system of rattlesnakes, and therefore the biological role of the jaw apparatus of *H. chlorophaea* is not as a venom system, or if a "venom system" it is much less capable of quickly dispatching prey (Kardong, 2002). These differences help account for why rattlesnakes equipped with a true venom apparatus (*C. oreganus* and *S. miliarius*) show a preference for mouse odors, and *H. chlorophaea* without a comparable venom system actually shows an aversion to mouse odor. Rattlesnakes have the venom system to exploit rodent prey or defend against them, *H. chlorophaea* do not.

While our study focused on three factors (shelter, prey, and temporal variables) effecting activity patterns in *H. cholorphaea*, such activity patterns in snakes may vary in response to several other factors as well. For instance, activity in small, nocturnal snakes such as *H. chlorophaea* could also be influenced by factors such as moonlight. However, most work conducted on snakes addressing any such factors has been on larger species, primarily viperid snakes (Yamagishi, 1974; Clarke et al., 1996; Theodoratus and Chiszar, 2000). Our laboratory study extends our knowledge to small colubroids by showing an endogenous rhythm in *H. chlorophaea* with shelter and time of day being important correlates with activity patterns and use of microhabitat.

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Specimens of *H. chlorophaea* and all native prey items were collected under Washington State Department of Fish and Wildlife Scientific Collecting Permit #06-253.

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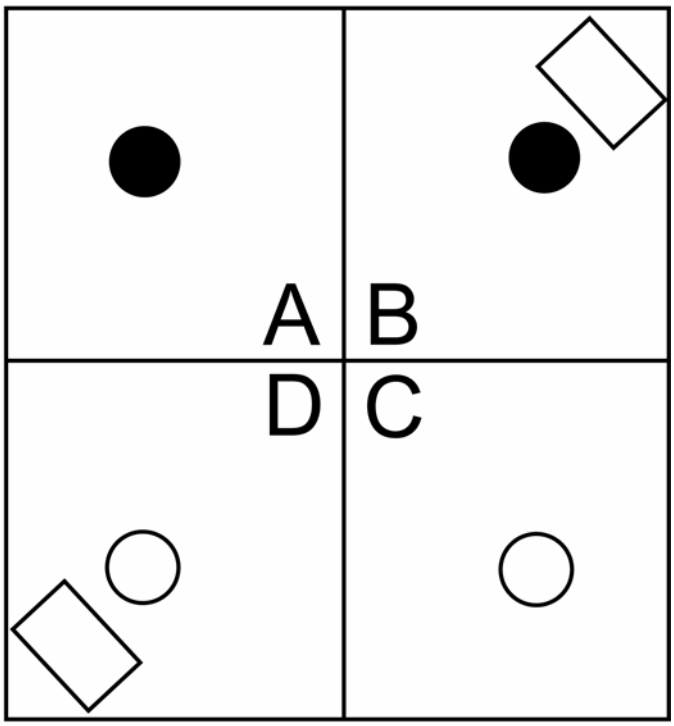
Fig. 1. Test Arena. (a) Experiment one. For each of the four quadrants A–D, a choice was provided—A: no shelter, prey odor; B: shelter,prey odor; C: shelter,no prey; D: no shelter,no prey odor. (b) Experiment two. An odor was provided in each of the four quadrants A–D—A: Mouse (M), B: Snake (S), C: Lizard (L), D: water, plus a shelter in each quadrant. The four odor/shelter combinations were changed and positioned at random during each of the trials. Circles, petri dishes with prey odor (closed circles) or water (open circles); rectangles, shelters.

Fig. 2. Total amount of time (minutes) spent in quadrants for all snakes during each 23 h trial for Experiment one (shelter and odor choices). Standard deviations are at the top of each bar.

Fig. 3. Total amount of time (minutes) spent in quadrants for all snakes during each 23 h trial for Experiment two (prey odor preferences). Standard deviations are at the top of each bar.

Fig. 4. Activity patterns. Average number of movements for all snakes per hour during the 23 h period. A–C show activity patterns for Experiment one for each of the three prey odors—lizard, snake, mouse. D, shows activity patterns for Experiment two, where all three prey odors and water were presented simultaneously.

a



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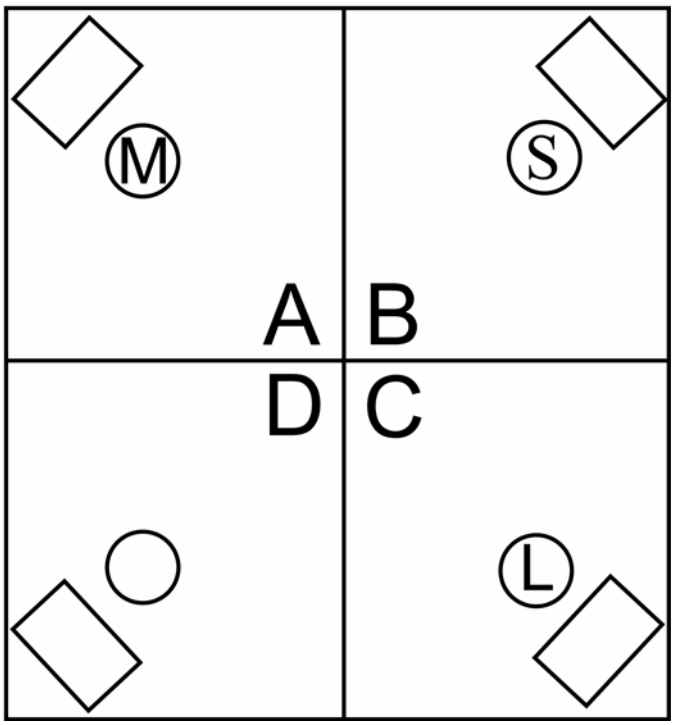

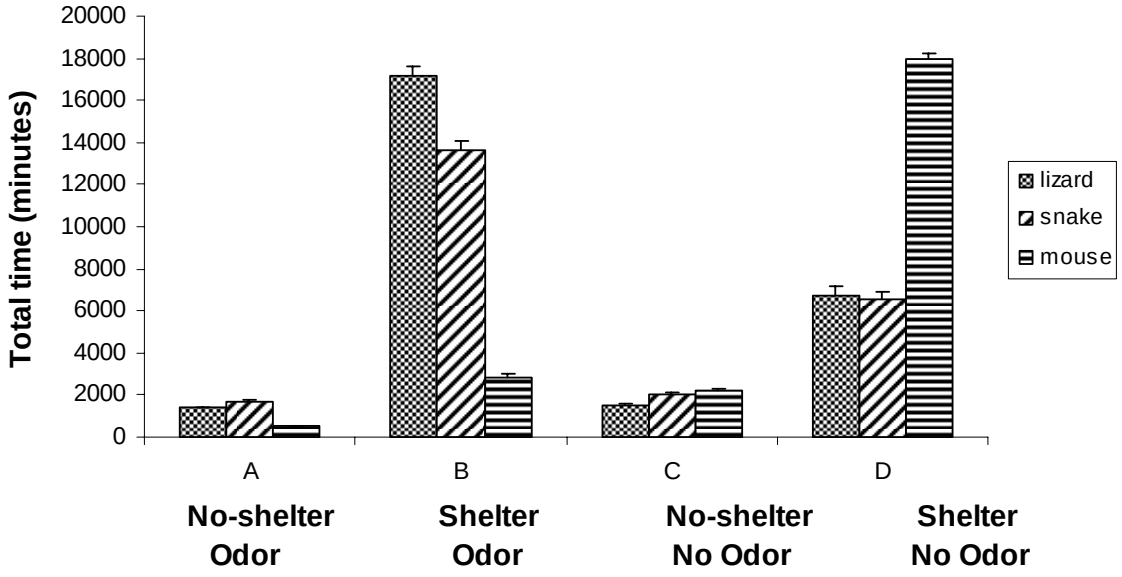
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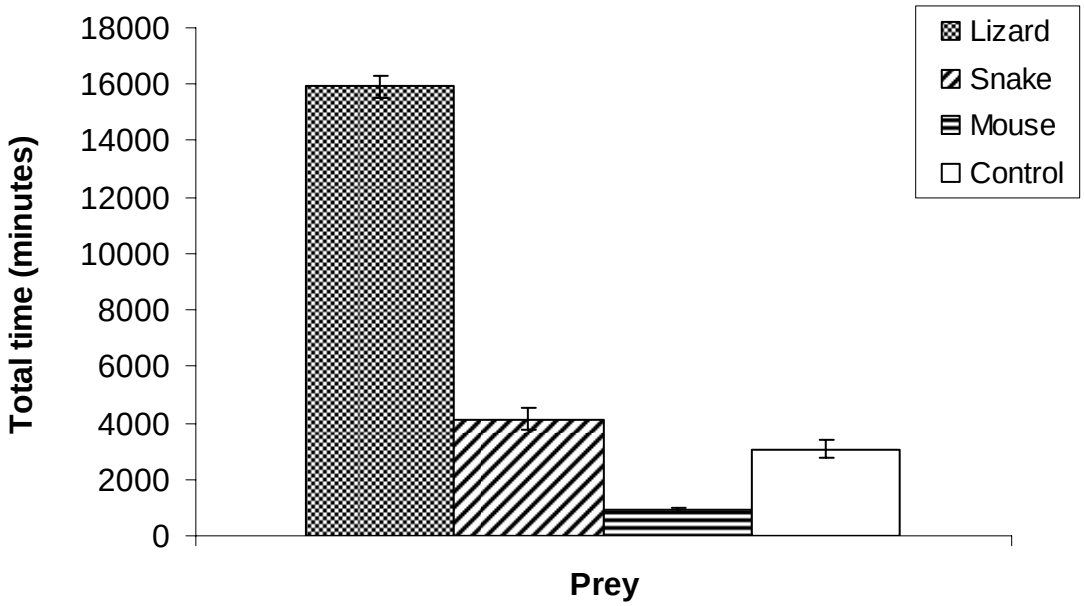
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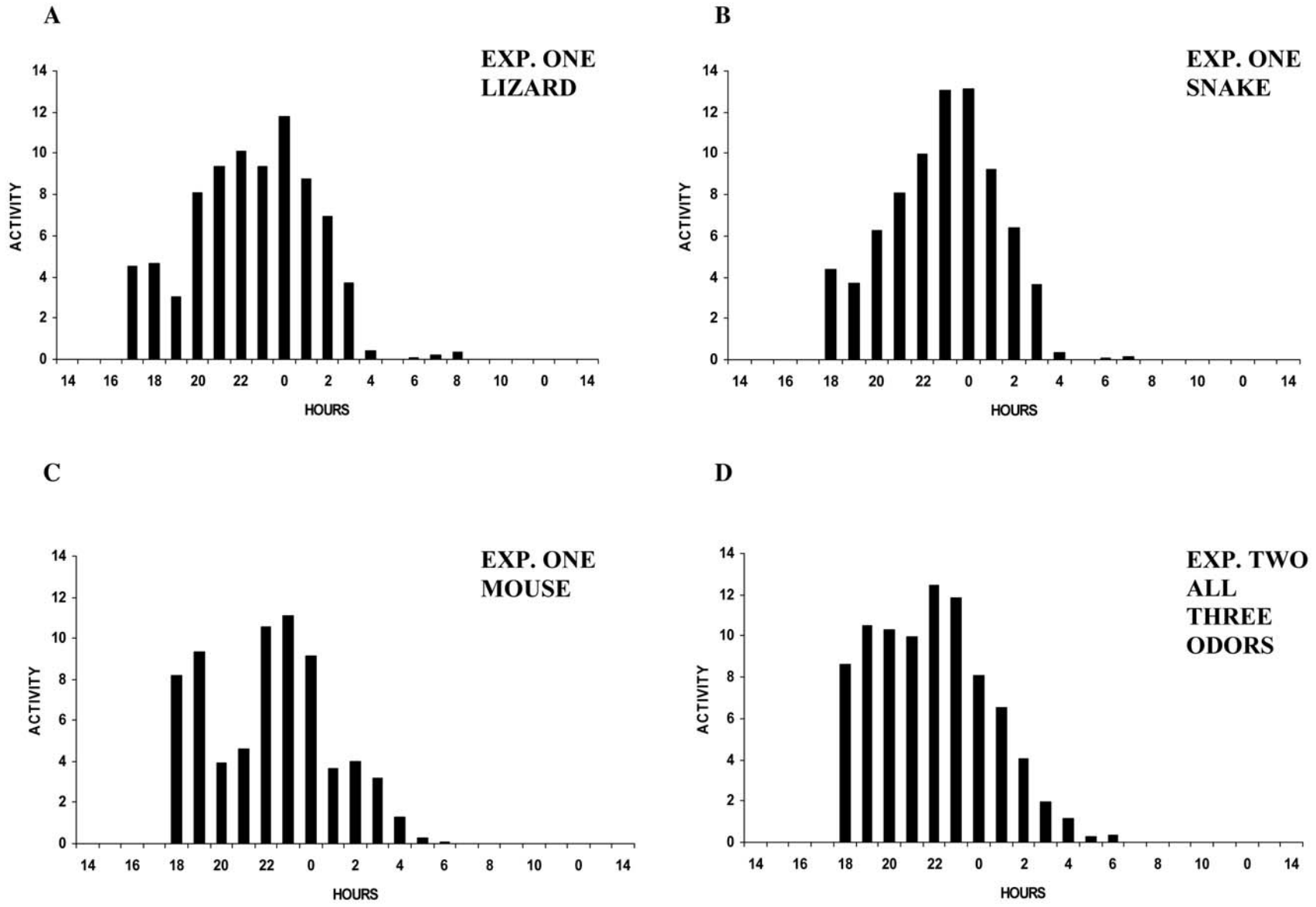
Mouse
Control


A
EXP. ONE
LIZARD
B
EXP. ONE
SNAKE
C
EXP. ONE
MOUSE
D
EXP. TWO
ALL
THREE
ODORS
ACTIVITY
HOURS
0
2
4
6
8
10
12
14
16
18
20
22

1

Table 1. Shelter-Site and Prey Odor Selection During 23-h Trials. **A: No shelter/Odor; B: Shelter/Odor; C: No shelter/No Odor; D: Shelter/No Odor. *Significant at α = 0.05. NS (not significant). Results of pair-wise multiple comparisons (Tukey test) in parentheses.**

	Lizard				Snake				Mouse			
	A	B	C	D	A	B	C	D	A	B	C	D
A	—	0.050* (5.822)	NS (0.506)	NS (3.227)	—	0.050* (5.347)	NS (0.158)	NS (2.531)	—	0.050* (3.702)	0.050* (3.923)	0.050* (7.625)
B	—	—	0.050* (5.315)	NS (2.594)	—	—	0.50* (5.189)	NS (2.816)	—	—	NS (0.721)	0.050* (3.923)
C	—	—	—	NS (2.721)	—	—	—	NS (2.373)	—	—	—	0.050* (3.702)

Table 2. Prey Odor Preference During 23-h Trial. ***Significant at α = 0.05. NS (not significant). Results of pair-wise multiple comparisons (Tukey test) in parentheses**

	Lizard	Snake	Mouse	Control
Lizard	—	NS	0.050*	0.050*
		(3.332)	(6.106)	(3.797)
Snake		—	NS	NS
			(2.784)	(0.475)
Mouse	—	—	—	NS
				(2.310)

CHAPTER TWO

Effects of simulated moonlight on activity patterns of a temperate dipsadine snake, the desert nightsnake (*Hypsiglena chlorophaea)*

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9 pages of text, 1 table, 1 figure

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Abstract

I tested the effects of simulated moonlight on 20 desert nightsnakes (*Hypsiglena chlorophaea*) collected from May–August 2008 at a site in central Washington State, USA. Snakes were maintained in captivity using standard husbandry practices. Based upon moon light levels gathered at the collection site, snakes were tested over a 23 hour period under three moonlight trials, new moon (0.05 lux), half moon (0.32 lux), and full moon (2.10 lux). Simulated moon-up during the half moon and full trials was from 2300–0300 hour. I detected no significant difference in the number of movements during either the new or half moon trials. However, snakes made significantly fewer movements from 2300–0300 hour (moon-up) during the full moon trials. For nocturnal species such as *H. chlorophaea* lower activity levels in response to a full moon may effect foraging time and patterns, mate searching behaviors, as well as movements to and from hibernacula. Alternatively, by decreasing activity during periods of bright moonlight, snakes may reduce the risk of predation.

Keywords *Hypsiglena chlorophaea* · Nightsnake · Moonlight · Activity Patterns

Introduction

Many factors affect the activity patterns of vertebrates. Biotic factors that have an effect on activity include, the sex or size of an individual (Andrews et al. 2009), age (Todd and Winne 2006), and reproductive condition (Schmidt et al. 2009). Additionally, the presence of potential predators (Eifler et al. 2008), or prey abundance (Zarybnicka 2009) may also affect activity. Animals shift or adjust activity due to several abiotic factors as well. Daily and seasonal temperatures (Sears 2005), and patterns of precipitation (Beltran and Delibes 1994) may all have an effect on activity.

Squamate reptiles are ectotherms (Zug et al. 2001), and as a consequence, shifts in activity in response to temperature can be acute and occur on a daily basis (Kerr at al. 2008), or gradual based on seasonal changes (Brown et al. 2002). Precipitation may have a stronger influence in geographic areas where patterns may be sporadic or strongly seasonal (e.g. deserts and tropical dry forests). The effects such abiotic factors have are mitigated by reptiles through behavioral responses such as thermoregulation (Huey and Benning 2001) and microhabitat selection (Whitaker and Shine 2002; Beck and Jennings 2003).

Other abiotic factors such as wind, humidity, and lunar phase may also have an effect on snake activity (Sun et al. 2001). This lunar abiotic factor has been shown to influence activity in some species of snakes. Adult prairie rattlesnakes (*Crotalus viridis*) may shift activity in response to moonlight (Clarke et al. 1996). Brown tree snakes (*Boiga irregularis*) are also known to reduce activity in response to bright (e.g. full moon) light (Campbell et al. 2008). Fish-eating snakes (*Lycondontomorphus bicolor*) forage less frequently under a full moon (Madsen and Osterkamp 1982). To see if moonlight effects activity patterns of a strictly nocturnal species of

snake, I chose to test the effects of simulated moonlight on the desert nightsnake (*Hypsiglena chlorophaea*).

Hypsiglena chlorophaea is a small (usually < 60 cm in total length), secretive and nocturnal snake distributed from the desert southwest and intermountain western United States northward into the Okanagan Valley of south-central British Columbia, Canada (Mulcahy 2008). The northern one-half of the range of *H. chlorophaea* encompasses southern Idaho, eastern Oregon, and central Washington (Nussbaum et al. 1983) where it occurs in a variety of habitats, including shrub-steppe dominated by big sagebrush (*Artemisia tridentata*), Oregon white oak (*Quercus garryana*) savannah, and Douglas-fir (*Pseudostuga meinziesii*) and ponderosa pine (*Pinus ponderosa*) forests (St. John 2002).

Historically, *H. chlorophaea* has been considered not just a secretive species, but rare in Washington State. However, recent field work has shown that *H. chlorophaea* is common and abundant in Washington State (Weaver 2008). Additional research on *H. chlorophaea* has shown its activity patterns to be entirely nocturnal (Weaver and Kardong 2009) and observed less often in the field during periods of a full or near full moon (Weaver in press).

Materials and methods

Twenty adult individuals of *Hypsiglena chlorophaea* were collected from May–August, 2008 at a site in south central Washington State, U.S.A. Of these 20 snakes, 11 were males (Mean SVL = 288 mm; range 240–334 mm), and nine were females (Mean SVL = 364 mm; range 332–502 mm). Snakes were housed individually in 26 x 51 cm glass aquaria, and maintained on 12:12 light cycle year around (lights on at 0830 hour and off at 2030 hour). Temperatures in both the

rooms housing the snakes and where experiments were performed were held at 25–30° C. Snakes were fed natural prey items on a weekly basis, with water available at all times. Snakes were maintained in captivity for at least three weeks before beginning experiments.

I conducted my experiment using square testing arenas (1.25 m wide x 0.5 m high) constructed out of compressed fiberglass panel, resting on a metal platform 20 cm above the floor. An overhead light provided 12 hours of simulated daylight, while a 20 watt red, incandescent bulb was used during 12 hours of darkness. Trials were run for 23 hours with one hour for change over (from 1700 to 1800 hour) between individual snake trials.

Snakes were placed into the center of an arena, and kept under a small plastic cup. This was then lifted at the start of a trial, recording commenced, and we then exited the room. Behaviors were filmed with Panasonic cameras suspended over each arena and recorded with a Panasonic time-lapse VCR. The arena was divided into four equal quadrants each with a small, 15 x 8 cm plastic shelter. We scored a movement when a snakes head first entered a quadrant. This was done for each hour, during the 23 hour trials.

To simulate moonlight, a string of 16, 0.05 watt light bulbs were suspended above the arena. These lights were run from a rheostat to control light intensity. Natural moonlight outputs were measured in the field at the collection location for the snakes. Light output was recorded for a new moon (0.05 lux), a half moon (0.32 lux), and a full moon (2.13 lux) using a standard 90% white card and a hand held digital light meter (Lodestar model LS1330A, Shenzen Inc., Hong Kong). Similar moonlight values and method of presentation has been used by previous researchers (Campbell et al., 2008). These recorded moonlight values were then simulated in the testing arena. Using known data on the activity patterns of *H. chlorophaea* (Weaver in press,

Weaver and Kardong 2009) simulated moon-up lasted five hours, during peak activity from 2300–0300 hour for both half and full moon trials.

Data were analyzed with PROC GLM (randomized complete block design, with a one-way treatment of structure) within SAS. When this test resulted in significance we used a Tukey post-hoc test procedure. All analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary NC, USA). Significances were determined at the level of $P \leq 0.05$.

Results

Snakes became active shortly after lights out at 2030 hour. During the three trials snakes made regular movements around the arena during each hour of darkness. Snakes investigated shelters, actively moving across open spaces and along walls. All snakes ceased movements from 0400–0500 hour.

The difference in movements between trials (treatments) during moon-up were significant ($F_{2,19} = 65.19$, $P < 0.0001$, Table 1). Post hoc analysis revealed these differences did not differ between full and half moon trials ($P = 0.0638$), but these trials significantly differed from the full moon trial ($P < 0.0001$). From 2030 until 2300 hour snakes moved 29.5 times (combined average movements) during the new moon trials and 24.8 times during the half moon trials. Snakes make nearly equal movements (24.3 times) during full moon trials (Fig. 1).

Simulated moon-up (half and full moon trials only) was during the next five hours (2300–0300 hours). Movement patterns during this time increased for both the new moon (44.4 movements), and half moon trial (39.5 movements). However, during the full moon trials

activity decreased to 22.75 movements (Fig. 1). Once our simulated moon had rose, snakes made rapid movements toward the nearest shelter, and most made no further movements.

Discussion

The results from my experiment show that a full moon does have an effect on activity in *H. chlorophaea*. Snakes reduced their activity by over 50% during the full moon trials compared to both half moon (57.5% less) and new moon trials (51.5% less). This response to increased light output of a full moon has the potential to reduced the possible risk of potential predation from mammalian or avian predators. Indeed, studies on the foraging of owls have shown that some species have greater success capturing prey during periods of increased moon light (Clark 1983). It is known that *H. chlorophaea* is an active forager (Weaver and Kardong 2009), which may increase encounter rates with predators. Natural predators of *H. chlorophaea* are not known. Given their small size, and lack of a protective venom system, any nocturnally active mammal or bird if inclined to do so, could potentially capture and consume this species.

This reduction in activity may also affect foraging time. Among nocturnal rodents a drop in activity in response to full moon (both simulated and natural) light intensities leads to a decrease in foraging time/success (Kotler 1984; Kramer and Biney 2001). It has also been shown that in at least one species of nocturnally foraging plethodontid salamander, foraging time is reduced under dim to bright light conditions (Placyk and Graves 2001). A decrease in foraging success has also been shown in an African snake, *Lycodontomophus bicolor*, where during a full moon the percentage of snakes encountered with prey in their stomachs dropped from 45% to 6% (Madsen and Osterkamp 1982). Our results, coupled with previous field research showing *H.*

chlorophaea is encountered less often in the field during periods of moderate to high moonlight (Weaver in press) indicates the effect on foraging time may be significant.

A full moon last just 24 hours, however a waxing gibbous moon could produce high enough amounts of illumination to have an effect on activity. Similar research on *Boiga irregularis* reported that when prey are available, *B. irregularis* avoided open spaces during a simulated full moon (Campbell et al., 2008). Instead, individual *B. irregularis* remained secluded in a simulated natural microhabitat (the foliage of a shrub).

Hypsiglena chlorophaea may also engage in such behavior, choosing to stay secluded among rock crevices, or outcrops. Snakes may move between such microhabitat while avoiding open areas. Field observations of such movements are lacking for *H. chlorophaea*, whose small body masses currently preclude implantation of transmitters. Unlike *H. chlorophaea*, *B. irregularis* occasionally makes diurnal movements (Tobin et al. 1999). Thus, moonlight may affect activity less so in *B. irregularis*, than in *H. chlorophaea*.

In an arena, edges may offer such a sense of seclusion. This has been shown with another species of snake, *Crotalus viridis*. During a simulated full moon, adult (but not juvenile) *C. viridis* moved more often along the edge of the testing arena and avoided open spaces (Clarke et al. 1996). I did not partition our observations of movements as either in the open or along the edge. In each trial, snakes moved very little (if at all) during a simulated full moon. Those few snakes that made movements did so quickly between adjacent shelters.

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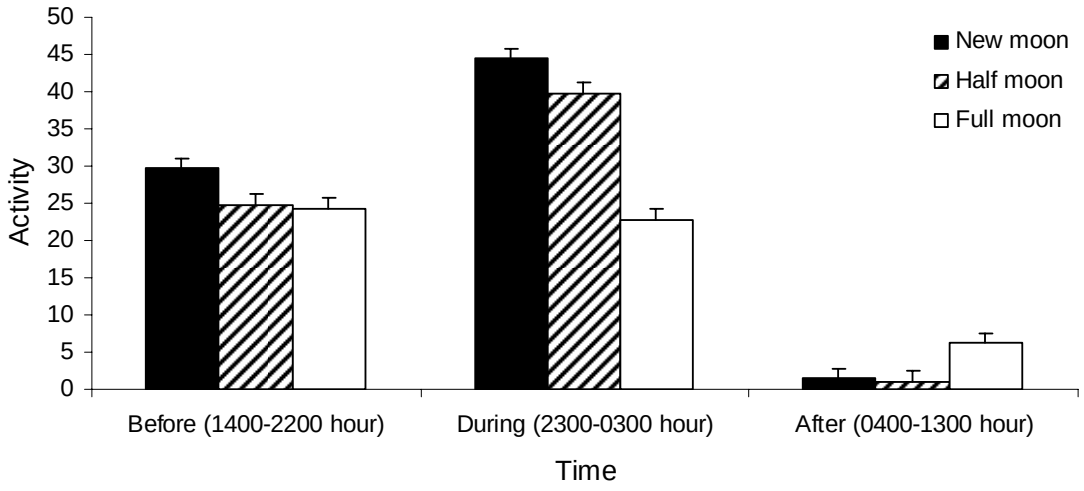
Table 1. Results from a randomized complete block design (PROC GLM) testing for differences in activity patterns of the desert nightsnake (*Hypsiglena chlorophaea*) during 2300–0300 hour for three simulated lunar phases (full, half, and full).

	df	Mean square	F	P
Snake	19	140.62	3.53	<0.0005
Treatment	2	2597.81	65.19	< 0.0001

Figure legends

Fig. 1 Activity patterns (total number of movements, ± 1 SE before, during, an after moon-up) for adult *Hypsiglena chlorophaea* during a 23 hour period for three separate trials of simulated moonlight (new, half, full). Simulated moon-up was during half and full moon trials from 2300–0300 hour.


50
45
40
35
30
25
20
15
10
5
0
Activity
New moon
Half moon
Full moon
Before (1400-2200 hour)
During (2300-0300 hour)
After (0400-1300 hour)
Time

CHAPTER THREE

Odor cues allow the desert nightsnake, *Hypsiglena chlorophaea* (Colubridae: Dipsadinae) to assess prey size

Running header: *Hypsiglena* and prey size discrimination

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[Formatted for *Behavioral Ecology*]

Odor cues allow the desert nightsnake, *Hypsiglena chlorophaea* (Colubridae: Dipsadinae) to assess prey size

Running header: *Hypsiglena* and prey size discrimination

Abstract

We whether desert nightsnakes (*Hypsiglena chlorophaea*) can chemically discriminate between two prey size classes (small and large) of the same prey species. Twenty-one adult individuals of *H. chlorophaea* (mean snout-vent length, SVL = 364 ± SD) were collected in 2008 from a site in Washington State, U.S.A. We obtained odors assays from a known prey item, the western terrestrial gartersnake (*Thamnophis elegans*) collected at the same site as *H. chlorophaea*. The size classes consisted of a small *T. elegans* (164 mm SVL) and a large *T. elegans* (640 mm SVL). We presented all odors on 15-cm cotton swabs held 2.5 cm in front of snake's snout. For each trial we recorded the number of tongue flicks in 60 seconds, and the latency to first tongue flick. We then compared individual snake responses to each prey size class, as well as to odor controls (water and cologne). Our analysis showed no statistically significant difference in latency times when comparing cologne to water, or comparing responses to small snake odor to these controls. Snakes performed tongue flicks most frequently in response to the small snake odor. Our study is the first to show that a species of snake can chemically discriminate between sizes of prey.

The ability for snakes to consume large prey items relative to their size is without equal among the vertebrates. Snakes are gape-limited predators, whose highly kinetic skulls (Kardong 1977) allow for incredible feats of ingestion. Snakes have been recorded to consume prey, upwards of 154% to 172% of their body-mass (Branch et al. 2002; Mulcahy et al. 2003). However, typical prey mass values are much lower than this. Snakes of all families have been recorded consuming a wide range of prey items, such as centipedes, arachnids, fish and their eggs, gastropods, small mammals, turtles, crocodilians, and other snakes (Greene 1997). The foraging strategies of snakes are equally diverse, and can be placed along a continuum from ambush to widely foraging predators (Arnold 1989). Both diet and foraging strategies can vary ontogenetically (Godley 1980; Mushinksy et al. 1982; Shine et al. 2006), seasonally (Madsen and Shine 1996; Shewchuk and Austin 2001; Hirai 2004), and between sexes (Shine 1991; Shine et al. 1998).

Using the classification of Greene (1983), prey items ingested by snakes have been shown to fall into one of four categories (type I, II, III, and IV) based upon the overall size and shape of prey. Type I prey are usually small in both mass and diameter (e.g. most athropods). Type II prey are considered to be long, and high in mass (e.g. anguiliform fish, other snakes), while Type III are equally high in mass, round in shape but not elongate (e.g. small rodents). Type IV prey are somewhat bulky relative to a low mass (e.g. birds).

Because snakes are gape-limited predators, the ability to consume large prey attributable to several key skull components (e.g. palatine-pterygoid arch, maxilla, quadrate) working in concert during the ingestion phase (Kardong 1977). Indeed, it has been shown that evolutionary forces acting on the skull of snakes results in changes in the size and shape of these individual components (Vincent et al. 2006). These have a concomitant effect on diet and prey handling behavior of snakes (Mori and Vincent 2008). Overall, the functional morphology of the skull and

dietary ecology are key forces in the evolution of snakes (Greene 1983; Rieppel 1988; Cundall and Greene 2000).

One other key component in the evolution of foraging in snakes is chemoreception. The highly modified tongue of snakes (Schwenck 1994), allows for the detection of favorable foraging sites (Clark 2004; 2007), as well as for location of prey (Kardong and Smith 2002). In many studies, snakes have been shown to discriminate based upon odor, both invertebrate and vertebrate prey (Greenbaum 2004), and different life-stages of vertebrates (Cooper and Secor 2007).

We tested whether the desert nightsnake (*Hypsiglena chlorophaea*) possesses the ability to discriminate through chemoreception. *Hypsiglena chlorophaea* is a small (usually < 66 cm total length), nocturnal snake, found throughout much of the western United States (Stebbins 2003). This species is generally considered to be a specialist and feed primarily on lizards, and their eggs (Rodriguez-Robles et al. 1999). Recent work on the diet of the desert nightsnake has shown it is very diverse. In addition to consuming lizards and their eggs, tree frogs, toads, snakes, and small mammals are also consumed (Weaver 2010), with recent laboratory experiments showing that desert nightsnakes engage in both sit-and-wait (ambush at a shelter) and wide-foraging predatory behavior (Weaver and Kardong 2009).

MATERIALS AND METHODS

Collection and maintenance of snakes

Snakes were collected from May–September 2008 from the Yakima River Canyon, located in two adjacent counties in central Washington State, U.S.A. Twenty-one adult *H. chlorophaea* (mean SVL = 364 mm ± 14.3 SD) were collected and housed individually in glass aquaria (26 x 31 x 51 cm), lined with paper. The room snakes were housed in was kept on a 12:12 light:dark cycle, at a relatively constant temperature of 28°C, with water available ad libitum. Snakes were deprived of food at least one week prior to testing. Individuals were maintained in captivity for at least three weeks prior to testing.

Behavioral experiments

The cage in which each snake was maintained in was utilized as the testing arena. We recorded the responses of individual snakes to chemical stimuli by presenting odors on 15-cm wooden, cotton-tipped swabs. We recorded latency period (time before first tongue flick) and the total number of tongue flicks exhibited by a snake for 60 seconds after the first recorded tongue flick. For our experiments we measured the above responses by snakes to four conditions. First was a control trial, which consisted of dipping a cotton swab into demineralized water. The second was a pungency control: A cotton swab was dipped into 3:1 mixture of water and commercial cologne (Aqua Velva brand). The third was odor obtained by rubbing a moistened cotton swab along the head, neck and mid-body region of a juvenile (SVL = 164 mm) western terrestrial

garter snake (*Thamnophis elegans*), which is known to occur naturally in the diet of desert nightsnakes (Weaver 2010). The fourth trial was odor obtained from an adult female garter snake (SVL = 640 mm) in the same way as that for juveniles.

Trials were begun through careful removal of the lid the a cage housing a snake. If a snake showed any unnatural movements we allowed it to settle down before continuing with a trial. After this period, a swab with one of the four conditions described above was presented 10–15 mm anterior to the snout of a snake. The cotton swab was kept in front of the snout, even if the snake vigorously approached the swab, or backed away. If a snake reacted in such a manner as to rapidly move away from the swab, the trial was terminated and we retested these snakes at a later time. All trials were conducted at night during the peak activity period (2400–0100 h) for desert nightsnakes (Weaver and Kardong 2009). Our observations were made with the aid of a 20-watt red light. We allowed for 3–4 hours between trials for each individual snake. All odors were presented in a random manner.

Statistical analysis

We used a non-parametric statistical test, Kruskal-Wallis (*H*-test) to test for significance for latency period, and rate of tongue flicks. If this test resulted in statistical significance, we performed a Tukey Test (*Q*-score) test of multiple pair-wise comparisons post hoc to show which were significantly different from one another. For both tests, α was set at 0.05. Means are reported ± SE.

RESULTS

Latency

During all trial snakes responded with tongue flicks for each odor tested. No snake reacted in a manner that required re-testing. Latency to first tongue flick differed significantly ($H = 24.09$, df = 3, $P < 0.001$). Post-hoc test showed these differences were between water and large snake, small and large snake, and cologne and large snake odors. There was no statistically significant difference between scores for any other trials (Table 1). Latency times were highest when presented with water, followed by cologne trials, large and small snake odors (Figure 1).

Rate of tongue flick

In each trial mean rates of tongue flicks differed significantly (Kruskal-Wallis, $H = 72.64$, df = 3, $P < 0.001$). A post-hoc analysis (Tukey Test) revealed significant differences were between water and small snake, cologne and both large and small snakes, large and small snakes, as well as water and cologne (Table 2). When presented with water, mean tongue flicks/60 s was 10.90 ± 4.37. For the cologne trials mean tongue flick/60 s was 3.00 ± 1.44. For both adult snake and juvenile snake odor trials, mean tongue flick/60 s were 18.09 ± 8.30, and 52.86 ± 13.83, respectively (Figure 2).

DISCUSSION

Our data clearly shows that *Hypsiglena chlorophaea* has an ability to chemically discriminate between large (un-ingestible) and small (ingestible) prey. Individuals responded with lower latency times toward small snakes than large, and showed a statistically higher mean rate of tongue flicks. These responses suggest that the subsequent behaviors exhibited by foraging *H. chlorophaea* may have an effect in terms of time spent and choices made while foraging. Prior work has shown that *H. chlorophaea* is both an active foraged at times, an ambush predator (Weaver and Kardong 2009). If, when actively moving across the landscape in search of prey (typically lizards or small snakes), a scent trail land down by a large prey item (such as an adult *Thamnophis* spp) is encountered, individual *H. chlorophaea* may simply ignore, or avoid such a scent, and saving foraging time by not following the scent. Odor cues may also be important when selecting an ambush site. Rather than hiding under a rock awaiting the return of a prey item that even the largest individuals of *H. chlorophaea* could not subdue and ingest, snakes may choose to wait where odors are present of more favorably sized prey.

Such behavior is shown in other species of snakes. Blindsnakes (*Rhamphotyphlops* spp.) will actively follow those scent trails laid by ants most easily consumed (Webb and Shine 1992), and Downes (1999), showed that juvenile broad-headed snakes (*Hoplocephalus bungaroides*) will select shelters with odors associated with preferred prey (e.g. small geckoes). This is also true of smooth snakes (*Coronella austriaca*), which used chemical cues to locate preferred prey (Amo et al. 2004). So once a scent trail is recognized as a prey species, the next step would be the ability to distinguish if such a prey items is ingestible.

Unlike many other species of snakes which undergo ontogenetic shifts in prey type (and size) as they grow (Mushinsky and Lotz 1980; Holycross and Mackessy 2002; Holycross et al. 2002), *H. chlorophaea* show no such growth related shift in diet, with all size classes feeding on vertebrate prey of similar size (Weaver 2010). For those snakes exhibiting an ontogenetic shift, effective discrimination among prey sizes using odor cues could help snakes optimize energy gain (Forsman 1996). Larger snakes can ultimately ingest larger prey, but this is affected by the head to snout-vent length (SVL) ratio. Some snake pecies such as European adders (*Vipera berus*) who possess a wide head relative to short SVL, being able to ingest rather large prey (Forsman and Lindell 1993). In nearly all cases, however, such vipers have much larger head to SVL ratios than non-vipers of equal SVL, again allowing for ingestion of large prey (Pough and Groves 1983). However, in our study we did not address the importance of body size or head to SVL ratios; the individuals tested were all adults and differed little in SVL.

In addition to the actual physical ability to ingest large prey, the metabolic and energetic costs of ingestion may also have a role in determining prey choice by snakes. Some snake species have shown differences in terms of energetic costs between populations. In some California populations of the western terrestrial gartersnake (*Thamnophis elegans*), those that fed exclusively on slugs had a higher assimilation rate than those with a generalist diet (Britt et al. 2006). In this same species, staged predatory encounters with a preferred vertebrate prey type (a salamander) showed that the cost of attack and ingestion is less than 1% of the net energy gain (Feder and Arnold 1982). This has also been shown to be true for juvenile rattlesnakes (Cruz-Neto et al. 1999). It is unknown if the same is true for a more slender-bodied snake such as *H. chlorophaea*. However, such physiological considerations may be irrelevant if *H. chlorophaea* is simply unable to capture, let alone ingest large prey.

Depending on the species, the line that divides either "large" or "small" prey may be relative to the size of the snake. Greene (1983) showed that while some prey may be bulky, the mass may be low. Other potential prey may be streamlined and less bulky, but greater in mass. Such prey is usually consumed by either truly venomous snakes such as viperids, or large constrictors such as boids who can overcome the relative bulkiness or massiveness of prey. *Hypsiglena chlorophaea* is neither, and the effects of relative prey size are magnified.

The effects of relative prey size on the foraging behavior of snakes need to be expanded to include species other than viperids, boids, or colubrids. Like *H. chlorophaea*, many other species of snakes remained understudied. While other studies have shown snakes can chemically recognize size differences interspecifically (LeMaster and Mason 2002, Shine et al. 2003), ours is the first to show that size may also be discriminated between genera. Without additional testing of other species, it remains unknown how wide-spread such ability is.

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Figure Legends

Figure 1

Mean ± SD latency (time until first tongue flick) of responses of *H. chlorophaea* to control odors (water and cologne) and odors of two size classes of prey (small and large snakes).

Figure 2

Mean ± SD frequency of tongue flicks per minute for *H. chlorophaea* in response to control odors (water and cologne) and odors of two size classes of prey (small and large snake).

Table 1

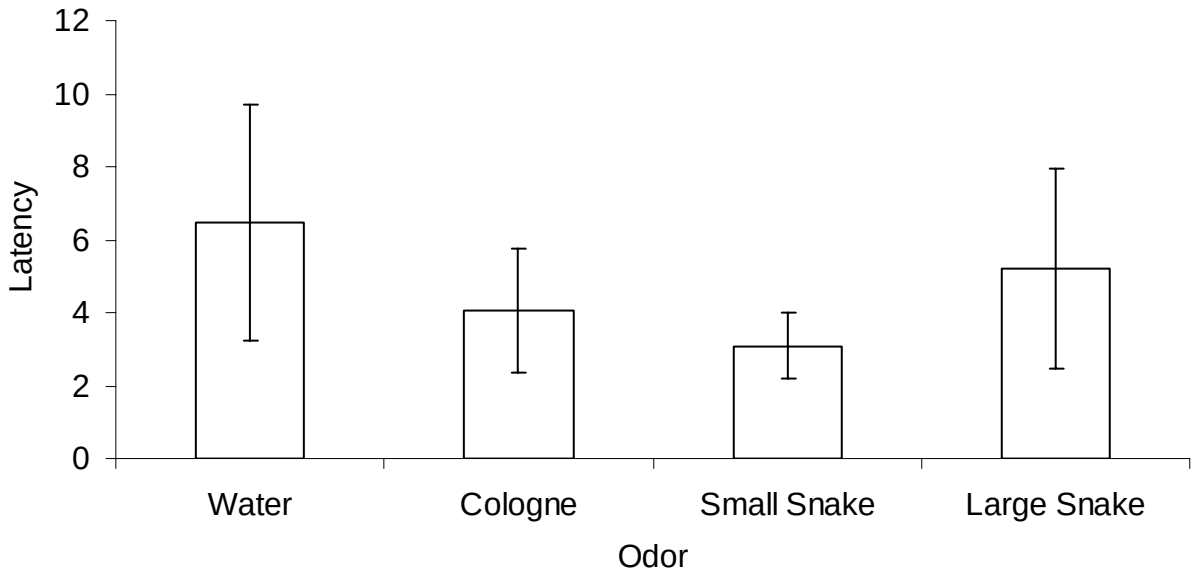
Results of Tukey Test (in parentheses): latency of *H. chlorophaea* responses to large and small snake prey odor and controls (NS = no significant statistical difference).

Stimuli	Water	Cologne	Large snake	Small snake
Water	—	NS (2.504)	<0.001 (6.685)	NS (2.262)
Cologne		—	<0.001 (4.181)	NS (0.242)
Large snake			—	<0.001 (4.424)
Small snake				—

Table 2

Results of Tukey Test (in parentheses): *H. chlorophaea* responses (rate of tongue flick/60s) to large and small snake prey odor and two controls. (NS = no significant statistical difference).

Stimuli	Water	Cologne	Large snake	Small snake
Water	—	<0.001 (4.694)	NS (2.207)	<0.001 (7.144)
Cologne		—	<0.001 (6.902)	<0.001 (11.838)
Large snake			—	<0.001 (4.936)
Small snake				—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10
8
6
4
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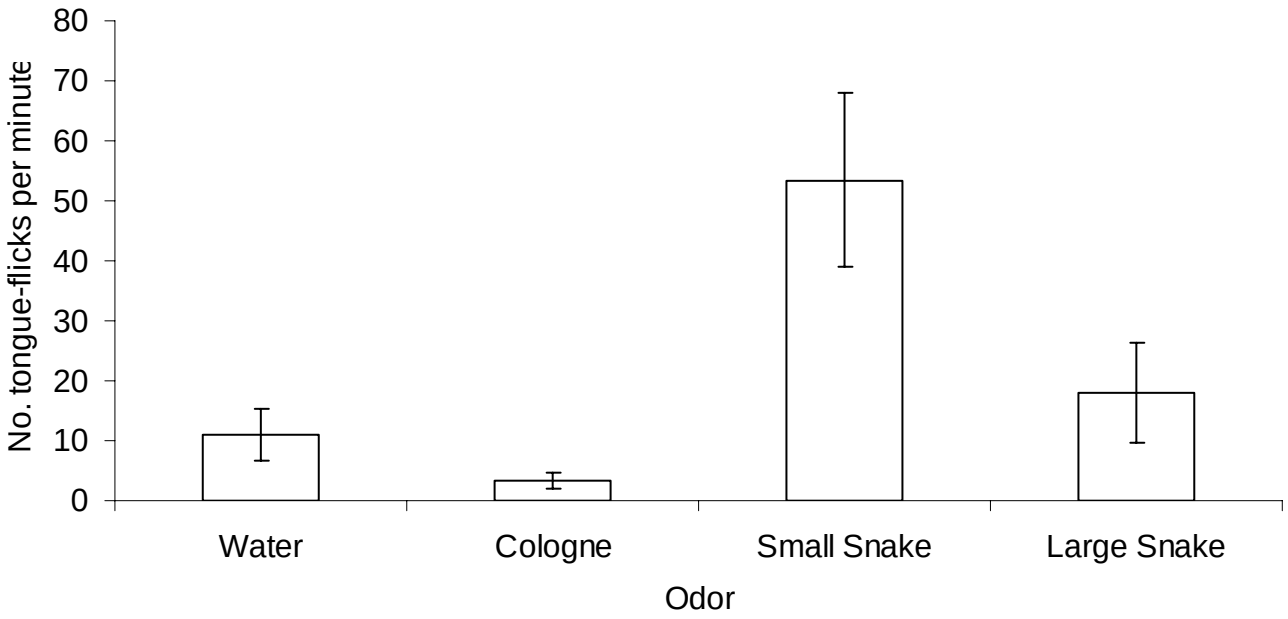
Latency
Water
Cologne
Small Snake
Large Snake
Odor


80
70
60
50
40
30
20
10
0
No. tongue-flicks per minute
Water
Cologne
Small Snake
Large Snake
Odor

CHAPTER FOUR

LRH: R. E. WEAVER AND K. V. KARDONG

RRH: *HYPSIGLENA* AND PREY ODOR DISCRIMINATION

Chemical Discrimination among prey by the Desert Nightsnake (*Hypsiglena chlorophaea*): Invertebrate and Vertebrate Prey Odor Cues

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ABSTRACT.—We investigated the responses of adult and juvenile Desert Nightsnakes (*Hypsiglena chlorophaea*) to odors of possible invertebrate and vertebrate prey. Snakes were collected during 2008 from three localities in Washington state. We obtained odor from three possible invertebrate prey: spider (*Tegenaria* spp.), scorpion (*Paruroctonus borealis*), and field cricket (*Gryllus* spp.), and compared responses to those toward a known vertebrate item (*Thamnophis* spp.). All prey items were collected at the same site as *H. chlorophaea*. We presented odors on 15-cm cotton swabs held 2.5 cm in front of snake's snout. For each trial we recorded the number of tongue flicks in 60 seconds, and the latency to first tongue flick. We observed no significant difference in tongue flicks or latency of responses between spider, scorpion, or cricket odors. Both adult and juvenile *H. chlorophaea* responded with a higher tongue flick rates to snake odor. Our study shows that *H. chlorophaea* does not exhibit a significantly different level of response toward the invertebrates species tested compared to odor controls. A result that is supported by previously published field work.

The chemical ecology of squamates has been well studied. Most of this research has been conducted on prey discrimination in scleroglossan lizard taxa such as gekkonids (Cooper, 1998), scincids (Cooper and Hartdegen, 1999), varanoids (Cooper and Arnett, 1995; Garrett et al., 1996), and pygopodids (Wall and Shine, 2009). Generally, non-scleroglossan squamates such as agamids and iguanids do not exhibit the ability to discriminate among potential prey items using chemical cues (Cooper, 2003).

The foraging ecology of a species plays an important role as to whether or not prey chemical discrimination abilities are present. This is especially true for snakes. Species that are known to be active foragers such as natricine (Burghardt, 1990; Krause and Burghardt, 2000) and colubrine snakes (Cooper et al., 1990; Cooper et al., 2000) are known to exhibit prey chemical discrimination. On the other hand, snakes that are considered ambush foragers such as viperids may not directly respond to prey chemical cues pre-strike (Lavin-Murcio and Kardong, 1995; Kardong and Smith, 2005). Instead these snakes appear to utilize a variety of chemical cues post-strike (Duvall et al., 1990; Smith et al., 2005).

The relative ease of collecting and maintaining snakes such as natricines and viperids has led to a disproportionate amount of research and more complete understanding of the chemosensory abilities of these taxa, while less common species are poorly understood. In orodr to better understand the prevalence of prey discrimination in snakes we chose to test the chemosensory abilities of a species of snake other than the groups previously mentioned. Specifically we investigated for the presence of prey chemical discrimination in a temperate dipsadine snake, the Desert Nightsnake (*Hypsiglena chlorophaea*).

The Desert Nightsnake is a small (usually < 66 cm snout-vent length) cryptic snake (Stebbins, 2003) found throughout the intermountain western United States, from Arizona (and northern Mexico) north to British Columbia (Mulcahy, 2008). Considered to be strongly saurophagus (Rodriguez-Robles et al., 1999), *H. chlorophaea* has been reported to occasionally feed on arthropod prey (Diller and Wallace, 1986; Werler and Dixon 2000). Some reports have indicated that juveniles of *H. chlorophaea* must feed solely on small invertebrates (Cowles, 1941). However, recent work on the ecology of this species in the northern half of its distribution has shown that both juveniles and adults to consume a wide range of vertebrate taxa (snakes, lizards, frogs, small mammals), but no invertebrates of any kind (Weaver, 2010). Using published dietary data and to either refute or support past claims of arthropods as prey, we investigated the behavioral responses of adult and juvenile *H. chlorophaea* to three possible invertebrate prey species against a known vertebrate prey species.

MATERIALS AND METHODS

Collection and Maintenance of Snakes.—Adult and juvenile specimens of *Hypsiglena chlorophaea* were obtained from May through September 2008 from two counties in central Washington State. Twenty-one adults (mean snout-vent length = 364 mm ± 14.3 SD) and 17 juvenile (mean = 164 mm ± 4.2 SD) were collected and housed individually in glass aquaria (26 x 31 x 51 cm), lined with paper. Age classes of *H. chlorophaea* were established using data from current field work on this species (Weaver, 2010). The room snakes were kept in was maintained on a 12:12 light:dark cycle, at a relatively constant temperature of 28°C, with water available ad libitum. To ensure hunger, snakes were not fed for at least one week prior to testing. Individuals were maintained in captivity for at least three weeks prior to testing.

Behavioral Experiments.—The cage each snake was maintained in was utilized as the testing arena. We recorded the responses of individual snakes to chemical stimuli by presenting odors on 15-cm wooden, cotton tipped swabs. We recorded latency period (time before first tongue flick) and the number of tongue flicks exhibited by a snake for 60 seconds after the first recorded tongue flick.

We measured the above responses by snakes to six odor stimulus. One was a control odor, which consisted of dipping a cotton swab into demineralized water. The second was a pungency control, in which a cotton swab was dipped into 3:1 mixture of water and commercial cologne (Aqua Velva brand). The third was an odor obtained from the extracts of a cricket (*Gryllus* spp.). The fourth was an odor obtained from extracts of a spider (*Tegeneria* spp.), and the fifth odor was obtained from extracts of a scorpion (*Paruroctonus borealis*). The sixth odor was collected from a known vertebrate prey item (Weaver, 2010) a juvenile Western Terrestrial Gartersnake (*Thamnophis elegans*). Following the methodology of Dial et al., (1989), extracts were prepared by grinding up 1.5 g of each invertebrate (ca. three or four individuals) in a test tube of 75 ml of demineralized water. Before dipping a cotton swab into a tube, suspended materials were allowed to settle. The snake odor was obtained by running a pre-moistened cotton swab tip along the anterior dorsal surface of the snake.

Trials were begun after lifting the lid of the cage housing the snake to be tested. If any snake reacted in adverse manner we allowed them to resume normal movements (or posture) before continuing with a trial. Snakes were then presented 10–15 mm anterior to the snout of a snake with a swab with one of the six conditions. The cotton swab was kept in this position even if the snake vigorously approached the swab, or backed away. If a snake moved away rapidly from the swab, the trial was terminated and we retested these snakes at a later time. All trials

were conducted nocturnally during a known peak activity period for *H. chlorophaea* (Weaver and Kardong, 2009). Observations were made with the aid of a dim, 20-watt red light. We allowed for two or three hrs to pass between trials for each individual snake, with odors presented in a random manner.

Statistical Analysis.— A non-parametric Kruskal-Wallis test (*H*-test) was used to compare overall responses to odors within each age group. If this test resulted in statistical significance, we performed a multiple pair-wise comparisons test (Tukey Test) post hoc to show which responses were significantly different from one another. For both, α was set at 0.05. We report means ± SD.

RESULTS

All individual snakes reacted to every odor with tongue flicks, and did not require retesting. For adult *H. chlorophaea* during all trials there was a significant difference in the latency periods ($H = 24.63$, $df = 5$, $P = 0.001$). The mean latency for water was 6.47 sec ± 2.35. For cologne it was 3.82 sec ± 1.67. For each of the four prey odors tested (snake, cricket, spider, scorpion), mean latencies were similar (Fig. 1). A post-hoc analysis revealed these differences in comparing mean latency times for adults was between water and each of the four invertebrate prey odors (not snake), but not between invertebrate prey odors (Table 1).

There was a statistically significant difference in the mean tongue flick rate of adults ($H = 91.75$, $df = 5$, $P < 0.001$). The mean tongue flick rate toward water was 9.00 ± 3.05; for cologne it was 3.09 ± 1.67. For each of the invertebrate odors tested mean tongue flick rates were similar (Fig. 2). Adult individuals responded with the highest number of tongue flicks to snake prey odor (53.47 ± 14.45). Post-hoc analysis revealed significant differences between responses to water

and cologne, water and each of the three invertebrate prey odors, and water and snake prey odor. There was also a significant difference between responses to snake prey odor and the three invertebrate odors, as well as water and cologne. There was no difference between the mean tongue flick rates in response to the three invertebrate prey odors, or between these odors and cologne (Table 1).

Overall, for each trial, juvenile *H. chlorophaea* also responded in a statistically significant manner with regards to latency ($H = 34.55$, $df = 5$, $P < 0.001$). Mean latency for water was 6.47 sec ± 2.26, and 3.82 sec ± 1.28 for cologne. For each of the four prey odors tested (snake, cricket, spider, and scorpion), latencies were nearly equal (Fig. 1). Post hoc analysis showed these differences were between water and cologne, water and each of the invertebrate prey odors and snake prey odor (Table 1).

The mean rate of tongue flicks for juvenile *H. chlorophaea* also differed in a statistically significant manner ($H = 73.19$, $df = 5$, $P < 0.001$). Mean rate of tongue flicks for water and cologne were 6.58 ± 1.88 and 2.52 ± 1.23, respectively. For each of the three invertebrate odors tested, mean rate of tongue flicks were very similar (Fig. 2), with juveniles responding the strongest to snake prey odors (47.47 ± 7.45). Post hoc analysis showed these differences were between water and cologne, and water and each of the invertebrate odors. This was also true when comparing snake prey odor to both controls and the three invertebrate prey odors (Table 2).

DISCUSSION

All individuals of *H. chlorophaea* tongue flicked during each trial and responded significantly more toward the vertebrate prey odor (small snake). However no snakes responded with a decreased latency or increased mean tongue flick rate to any of three invertebrate prey

odors (cricket, spider, and scorpion). In some trials, both adult and juvenile *H. chlorophaea* approached the swab with a spider or scorpion odor, tongue flicked briefly then quickly crawled away. This was especially true when presented with scorpion odor. In all cases, when compared to invertebrate prey odor, *H. chlorophaea* tongue flicked at a higher rate to the water control, and in a similar manner to cologne.

Such a reaction to these arachnids is not surprising. There are known cases of predation events by such terrestrial arthropods on snakes similar in size t *H. chlorophaea* (Greene, 1994; 1997). This is also true for other types of arthropods. Crustaceans such as crayfish (Weaver, 2004), marine and land crabs (Voris and Jefferies, 1995; Maitland, 2003) have all been reported to kill and consume snakes. In these latter cases, crabs killed species typically much larger than *H. chlorophaea*, such as *Cerebus rynchops* and *Oxybelis aeneus*. But, individuals that were predated were juveniles. In all other reports those individuals consumed were adults of species similar in size to *H. chlorophaea*, such as *Sibon nebulata* or *Atracus trilineatus*.

Given the small size of the spiders we collected odors from, we feel that they most likely pose little threat, even to the smallest juvenile (e.g. hatchlings) *H. chlorophaea*. However, some spiders, such as genera in the family Lycosidae reach sizes large enough to pose a possible threat to juvenile *H. chlorophaea*. The species of scorpion used reach an adult size that may enable them to capture and consume juvenile *H. chlorophaea*. Several individuals of *H. chlorophaea* reacted in a much more overt defensive manner upon tongue flicking towards scorpion odors. In other parts of the Pacific Northwest, scorpions such as *Hadrurus spadix* are much more robust, and could conceivably kill small adult *H. chlorophaea*. While such predation events are rarely documented, there is a report of a similar sized species of scorpion killing (but not consuming) a snake (*Leptotyphlops humilis*), albeit a species smaller than *H. chlorophaea* (Anderson, 1956). In

the southwestern United States, with its abundance of larger arachnids, such possible predation events on *H. chlorophaea* may be more likely (McCormick and Polis, 1982). With regard to cricket odors, snakes approached, tongue flicked for just a few seconds then simply ignored the odor. When the snake moved away the swab was repositioned, with snakes remaining uninterested. *Hypsiglena chlorophaea* of all sizes consume vertebrate prey, including small snakes (Weaver, 2010), hence the lower latency and substantially higher tongue flicks of both adult and juveniles toward this type of odor is not surprising. Our data contrasts with reports that juvenile *H. chlorophaea* are not capable of capturing and killing small lizards and snakes, and must therefore feed on invertebrates (Cowles, 1941; Werler and Dixon, 2000).

Abundance of invertebrate prey of all classes and types in the northern portion of the distribution of *H. chlorophaea* is undeniable. We did not formally test responses of *H. chlorophaea* to other classes (either adults or larvae) of terrestrial invertebrates (e.g. Coleoptera) and some authors have reported such invertebrates to be prey for these snakes (Diller and Wallace, 1986; Rodriguez-Robles et al., 1999). Since the lizard prey of *H. chlorophaea* are insectivorous, earlier reports of invertebrate prey for *H. chlorophaea* are most likely based on finding the remains of, or even whole invertebrates that have been secondarily ingested.

It cannot be overlooked that widely distributed species of snakes have a similarly broad diet. This is the case for natricines (Burghardt, 1993), colubrines (Cooper et al., 1990; 2000; Shewchuk and Austin, 2001), and viperids (Greenbaum, 2004). In some cases, species with populations separated by short distances (< 100 km) may have very different diets (Aubret et al., 2006; Weatherhead et al., 2009). *Hypsiglena chlorophaea* is found from northern Sonora and Baja California Mexico north into British Columbia (Mulcahy, 2008). It stands to reason that the

diet will vary geographically, and that populations of *H. chlorophaea* from the Sonoran Desert may feed on invertebrates.

Competition and prey availability both may play a role in the diet of snakes. Sympatric species of snake partition prey in response to an overlap of diet between species (Luiselli, 2006; Brischoux et al., 2009). We did not sample prey availability and whether or not this is a factor is unknown. Diets also shift in snakes in response to differences in prey availability across the landscape (Santos et al., 2000; Santos et al., 2006). At the site where we collected *H. chlorophaea* tested, it is the only small and entirely nocturnal species of snake feeding on squamate reptiles. This is true for most of its northern distribution. *Rhinochelius lecontei* is sympatric in a small part of southwestern Idaho and Nevada and is the only other snake that may compete for prey. So competition is most likely not an issue. It is possible that an open niche may allow northern *H. chlorophaea* to choose not to feed on invertebrates. This may not be the case throughout its southern distribution, where there are several other species of snakes with similar life-histories.

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Figure Legend

FIG. 1. Mean latencies (time to first tongue flick) ± SE for adult (N = 21) and juvenile (N = 17) *Hypsiglena chlorophaea* in response to odors of one vertebrate prey (a snake), two control odors (water and cologne) and three possible invertebrate prey odors (cricket, spider, scorpion).

FIG. 2. Mean rate of tongue flicks/60 sec ± SE for adult (N = 21) and juvenile (N = 17) *Hypsiglena chlorophaea* in response to odors of one vertebrate prey (a snake), two control odors (water and cologne) and three possible invertebrate prey odors (cricket, spider, scorpion).

TABLE 1. Results (in parentheses) of pair-wise multiple comparisons (Tukey Test) of latency (time to first tongue flick) toward two odor controls (water and cologne) three invertebrate (cricket, spider, scorpion) and vertebrate (snake) prey odors for 21 adult (mean SVL = 364 mm ± 14.3 SD) *Hypsiglena chlorophaea*. * Significant at α = 0.05. NS (not significant).

	Snake	Water	Cologne	Cricket	Spider	Scorpion
Snake	—	0.05 (6.460)	NS (2.695)	NS (1.904)	NS (1.580)	NS (1.642)
Water		—	0.05 (4.780)	0.05 (5.121)	0.05 (6.145)	0.05 (5.918)
Cologne			—	NS (0.341)	NS (1.366)	NS (1.138)
Cricket				—	NS (1.024)	NS (0.797)
Spider					—	NS (0.228)
Scorpion						—

TABLE 2. Results (in parentheses) of pair-wise multiple comparisons (Tukey Test) of mean tongue flicks toward two odor controls (water and cologne) three invertebrate (cricket, spider, scorpion) and one vertebrate (snake) prey odors for 21 adult (mean SVL = 364 mm ± 14.3 SD) *Hypsiglena chlorophaea*. * Significant at α = 0.05. NS (not significant).

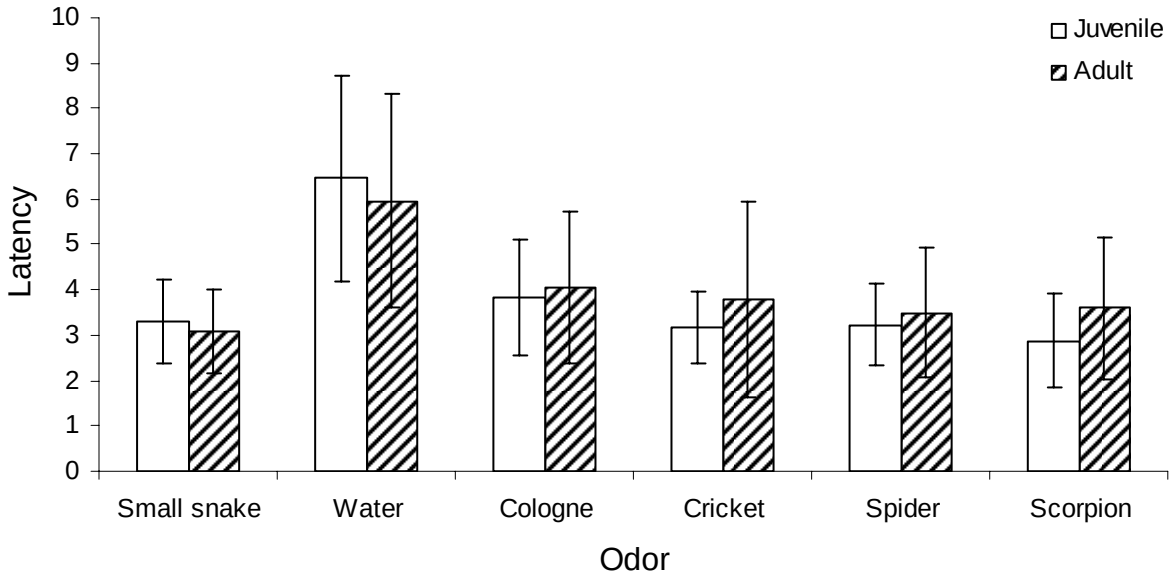
	Snake	Water	Cologne	Cricket	Spider	Scorpion
Snake	—	0.05* (8.033)	0.05* (9.094)	0.05* (8.121)	0.05* (9.972)	0.05* (10.345)
Water		—	0.05* (4.780)	0.05* (5.121)	0.05* (6.145)	0.05* (5.918)
Cologne			—	NS (0.341)	NS (1.366)	NS (1.138)
Cricket				—	NS (1.024)	NS (0.797)
Spider					—	NS (0.228)
Scorpion						—

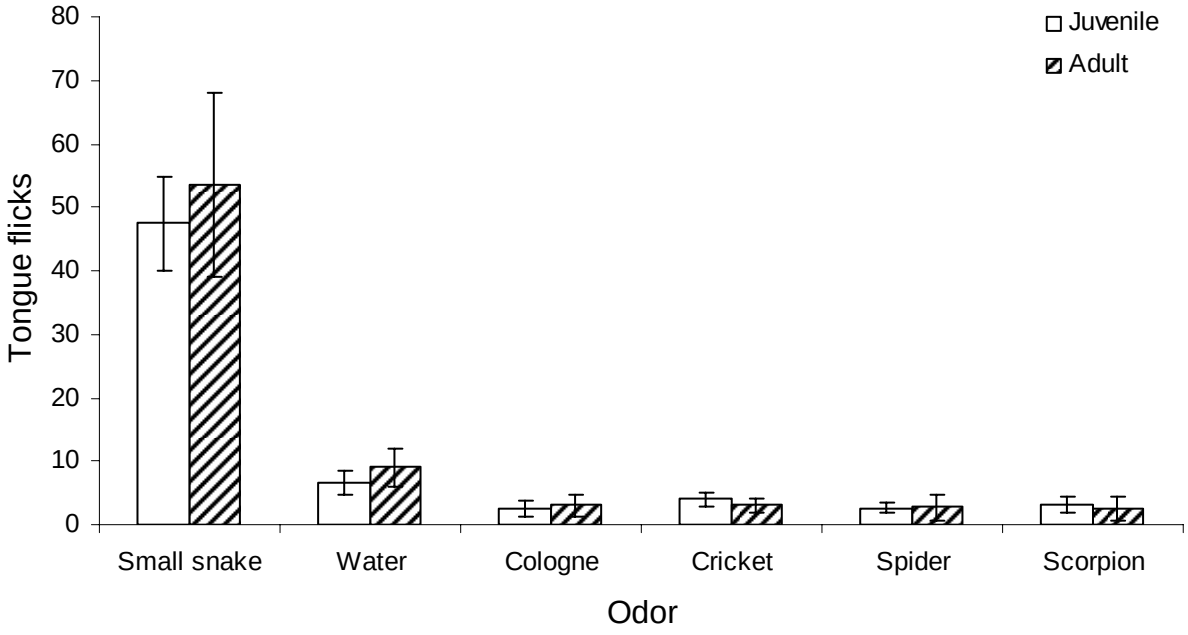
TABLE 3. Results (in parentheses) of pair-wise multiple comparisons (Tukey Test) of latency (time to first tongue flick) toward two odor controls (water and cologne) three invertebrate (cricket, spider, scorpion) and vertebrate (snake) prey odors for 17 juvenile (mean SVL= 164 mm ± 4.2 SD) *Hypsiglena chlorophaea*. * Significant at $\alpha = 0.05$. NS (not significant)

	Snake	Water	Cologne	Cricket	Spider	Scorpion
Snake	—	0.05* (5.607)	NS (1.328)	NS (0.627)	NS (0.266)	NS (1.393)
Water		—	0.05* (4.260)	0.05* (6.142)	0.05* (5.798)	0.05* (6.893)
Cologne			—	NS (1.882)	NS (1.538)	NS (2.634)
Cricket				—	NS (0.344)	NS (0.752)
Spider					—	NS (1.096)
Scorpion						—

TABLE 4. Results (in parentheses) of pair-wise multiple comparisons (Tukey Test) of mean tongue flick rates toward two odor controls (water and cologne) three invertebrate (cricket, spider, scorpion) and vertebrate (snake) prey odors for 17 juvenile (mean SVL= 164 mm ± 4.2 SD) *Hypsiglena chlorophaea*. * Significant at α = 0.05. NS (not significant)

	Snake	Water	Cologne	Cricket	Spider	Scorpion
Snake	—	0.05* (7.039)	0.05* (9.328)	0.05* (6.217)	0.05* (9.500)	0.05* (7.611)
Water		—	0.05* (6.451)	0.05* (5.553)	0.05* (6.623)	0.05* (4.734)
Cologne			—	NS (3.111)	NS (0.172)	NS (1.717)
Cricket				—	NS (3.283)	NS (1.393)
Spider					—	NS (1.889)
Scorpion						—


10
9
8
7
6
5
4
3
2
1
0
Latency
Juvenile
Adult
Small snake
Water
Cologne
Cricket
Spider
Scorpion
Odor


80
70
60
50
40
30
20
10
0
Tongue flicks
Juvenile
Adult
Small snake
Water
Cologne
Cricket
Spider
Scorpion
Odor

CHAPTER FIVE

BEHAVIORAL RESPONSES TO POTENTIAL PREY THROUGH CHEMORECEPTION BY THE SHARP-TAILED SNAKE (*CONTIA TENUIS*)

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ABSTRACT—The Sharp-tailed Snake (*Contia tenuis*) is a small (usually <30 cm total length), cryptic species found along the west coast of the United States and north into southwestern British Columbia. Because of its secretive nature, little is known about its behavioral ecology. We tested behavioral responses of 13 adult *C. tenuis* collected from a site in eastern Washington to potential invertebrate prey odors. We presented snakes with 2 control odors (water, cologne) and 2 possible invertebrate prey odors (earthworm, slug). Overall, there was a significant difference in both the time-to-first-tongue flick (latency) and mean tongue flick rate (number of tongue flicks/60 s trial) for the odors tested. The mean latency period was 6.0 ± 1.87 s for earthworm and 4.1 ± 1.57 s for slug. The mean tongue flick rate for earthworm and slug was 13.8 ± 4.09 flicks/s and 39.7 ± 15.79 flicks/s, respectively. These results support prior claims of a preference for slugs by *C. tenuis*. This preference for slugs may also explain the presence of *C. tenuis* in areas of anthropogenic disturbances with an abundance of slugs.

Key words: Sharp-tailed Snake, *Contia tenuis*, prey preference, chemoreception, slugs

Chemoreception plays an important role in several aspects of the behavioral ecology of vertebrates. For many vertebrate groups, the detection of prey is mediated through chemoreception (among other sensory modalities) and, in particular, for some groups such as squamate reptiles via stimuli conducted to the vomeronasal organ (Schwenk 1993). Such vomerolfaction is facilitated in snakes by a highly modified tongue (Schwenk 1988).

The chemical ecology of just a few groups of snakes is well known. Most studies have focused on species that are medium- to large-bodied and easily collected in large numbers, such as viperids (Kardong 1993; Roth and others 1999; Kardong and Smith 2002) or natricines (Krause and Burghardt 2001; Waters and Burghardt 2005). To date, very few chemoreception

studies have been conducted on small-bodied, cryptic species of snakes, and what is known is limited to studies on Australian elapids (Downes 1999, 2002). Such studies allow for insights into the behavioral ecology of these poorly known but often widespread species.

We investigated prey discrimination via chemoreception in a small (usually <30 cm total length) species of snake, the Sharp-tailed Snake (*Contia tenuis*). *Contia tenuis* is found along the west coast of the United States, from central California, north into western Oregon (and to a limited extent, eastern Oregon), northwestern and central Washington State, and southwestern British Columbia (St John 2002). Within this distribution, *C. tenuis* is found in a wide range of habitats, but is most often associated with oak savannah and open woodlands (St John 2002).

Based upon the association of *C. tenuis* with generally moist, cool habitats, anecdotal reports, and limited studies, *C. tenuis* is thought to feed primarily on slugs (Darling 1947; Cook 1960). Morphological evidence also suggests such a dietary predilection. The teeth of *C. tenuis* are narrow and strongly re-curved (Zweifel 1954; Britt and others 2009). It has been suggested that such teeth allow *C. tenuis* to grasp and swallow slippery prey such as slugs (Zweifel 1954). This preference for slugs has been referred to many times in regional field guides (Darda 1995), as well as more comprehensive guides (Stebbins 2003). However, no extensive dietary studies have been conducted. The few prey items (all slugs) that have been recorded have come from limited observations (Darling 1947). Furthermore, despite the abundance of *C. tenuis* in some parts of its range (Hoyer and others 2006), no studies have been conducted on the prey preference of *C. tenuis*. Therefore, we used the experimental design and statistical treatment established by Cooper (1989, 1994, 2003) to examine behavioral responses to likely non-prey and prey odors with the objective of detecting possible prey preferences in *C. tenuis*.

METHODS

Collection and Maintenance of Snakes

Thirteen adult specimens of *C. tenuis* (7 females and 6 males, mean snout-vent length ± SD = 224 ± 19.5 mm) were collected from a site in central Washington State (approximately 9.2 km WNW of Ellensburg, Kittitas County). Individual snakes were maintained in 26 x 31 x 51 cm glass aquaria with a peat moss-mulch bedding 15 mm deep. The snakes were kept in a room with a 12 h:12 h light:dark cycle and a relatively constant temperature of 28°C. Water was available *ad libitum*. Individuals were maintained in captivity for 1 mo prior to testing, and were not fed during this period. Prey items used during testing were earthworms (*Eisenia* spp.) and non-native slugs (*Arion* spp.) collected at the same locality as the snakes.

Behavioral Experiments

Each snake was tested in its cage to an odor presented on a 15-cm long cotton swab. We recorded the latency period (time in seconds from the presentation of the cotton swab to 1st tongue flick) and the tongue flick rate (number of tongue flicks exhibited by a snake during 60 s after the 1st recorded tongue flick). Four odors were presented, in random order, to snakes on a cotton swab dipped in the odor: 1) demineralized water; 2) a 3:1 mixture of water and commercial pungent cologne (Aqua Velva brand); 3) odor obtained by rubbing a cotton swab moistened with demineralized water along the surface of a live earthworm; and 4) odor obtained from live slugs in the same way as that for earthworms.

To begin a trial, we removed the lid to the cage housing a snake. If a snake showed any unnatural movements interpreted as stress escape, we waited until it again settled into a motionless posture before continuing with the trial. A swab with 1 of the 4 odors was presented 10 to 15 mm anterior to the snout of a snake. We scored the latency period and the tongue flick

rate. The cotton swab was held in front of the snake, even if the snake vigorously approached the swab, or retreated. If a snake reacted rapidly and moved away from the swab, the trial was terminated, the scores up to that point were not used, and the snake was retested at a later time. All trials were conducted during the nocturnal phase of the light and dark cycle (2000 to 2300) when *C. tenuis* has been observed to be active based upon field observations (Weaver 2002), and observations were made with the aid of a 20-watt red light.

Statistical Analysis

We used the non-parametric Kruskal-Wallis (*H*-test) statistical test to compare rates of tongue flicks and latency between odors. When this test resulted in statistical significance ($\alpha = 0.05$), we performed a Tukey Test (*Q*-score) of multiple pair-wise comparisons to identify which trials were significantly different from each other.

RESULTS

All 13 snakes responded to each odor presented by exhibiting at least some tongue flick activity. No snake attacked a swab during trials. There was an overall significant difference for latency to 1st tongue flick among all tests, snakes, and odors ($H = 34.11$, $df = 3$, $P < 0.001$). Post-hoc analysis detected statistical differences between water and earthworm, water and slug, cologne and earthworm, and cologne and slug, (Table 1; Fig. 1). Mean tongue flick rates also differed significantly ($H = 39.72$, $df = 3$, $P < 0.0001$). These differences were between water and slug, cologne and slug, and earthworm and slug (Table 1; Fig. 2).

DISCUSSION

Based upon these results, this population of *C. tenuis* does show a preference for slug odors. This supports prior claims made by authors of such a preference (Cook 1960; Darda

1995). The latency period for slug and earthworm odors did not vary significantly, and is a result of snakes responding to both odors as novel. Snakes responded with a significantly higher mean tongue flick rate to the preferred slug odor than to earthworm odor.

Slugs can be found in some of the arid portions of eastern Washington (Pearce and others 2004), allowing for *C. tenuis* to survive in such habitat usually considered atypical (Weaver 2002). Several introduced species of slugs (such as *Deroceras* and *Arion* spp., Gordon 1994) are present throughout the range of *C. tenuis*, especially in disturbed areas, including urban areas. The availability of abundant prey may account for the ability of *C. tenuis* to persist in such areas, despite anthropogenic disturbances (Spalding 1995; Weaver and Darda 2003). Stomach contents and fecal samples collected from nearly 100 individuals have revealed no identifiable annelid or arthropod prey items (Weaver, unpub. obs.). Such samples have consisted of dark, watery feces with no chitinous remains. These observations and our experimental result showing preference for slugs based on tongue flick rate support previous suggestions that *C. tenuis* feeds primarily on slugs rather than on other invertebrates.

It is possible that *C. tenuis* also feeds on terrestrial snails. Snakes that feed on snails, such as North American natricine snakes of the genus *Storeria* (Rossman and Myers 1990), often have highly specialized morphological and behavioral features that allow them to extract the prey from its shell. *Contia tenuis* possesses at least 1 morphological similarity to *Storeria* spp., needle-like teeth on dentary (Zweifel 1954), and so it is possible that *C. tenuis* also feeds on snails. Future research on the prey preference of *C. tenuis* should include snails, as well as other invertebrates.

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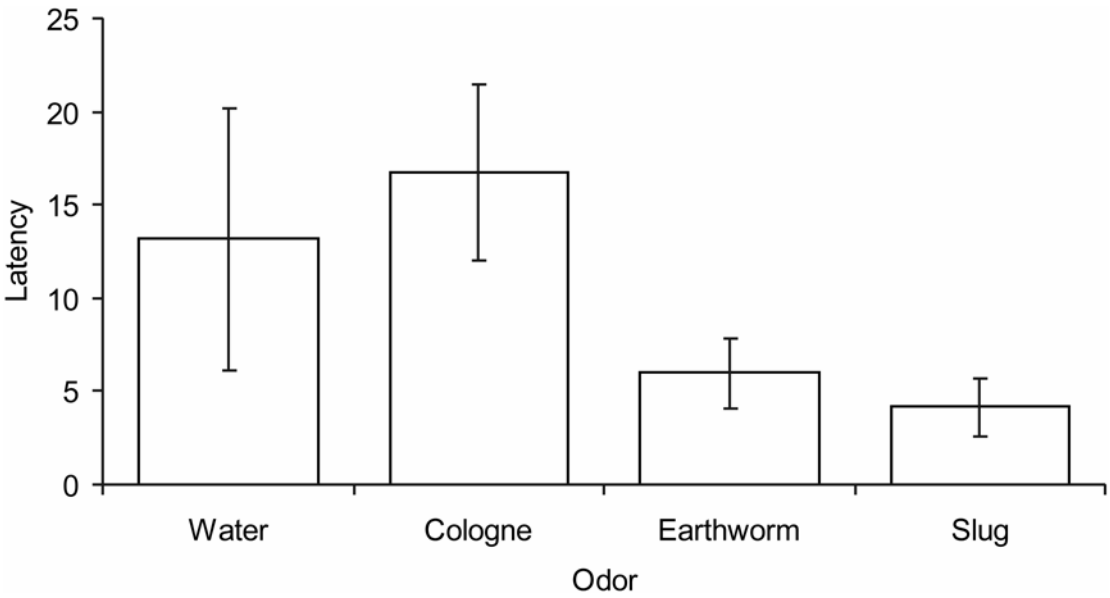
FIGURE CAPTIONS

FIGURE 1. Latency period (time to 1st tongue flick in seconds ± *s*) for adult *Contia tenuis* (*n* = 13) in response to control odors (water and cologne) and 2 potential invertebrate prey odors (earthworm and slug).

FIGURE 2. Mean number of tongue flicks (± *s*) during a 60 s trial for adult *Contia tenuis* (*n* = 13) in response to control odors (water and cologne) and 2 potential invertebrate prey odors (earthworm and slug). The 60 s period was measured from the 1st tongue flick


25
20
15
10
5
0
Latency
Water
Cologne
Earthworm
Slug
Odor

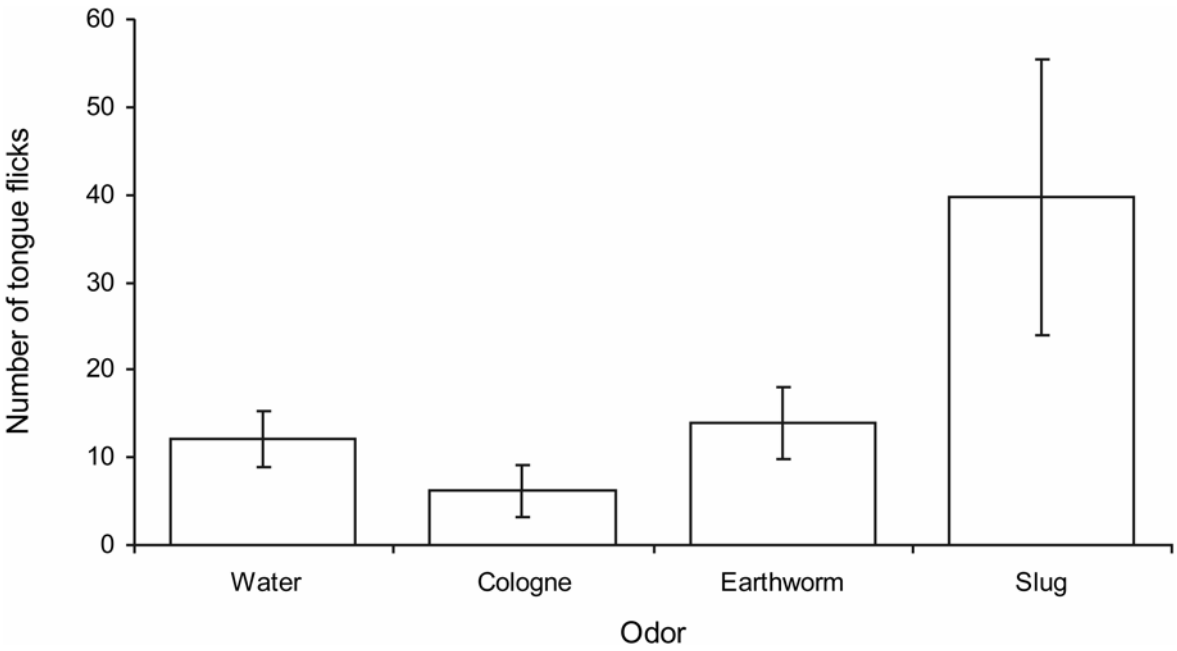

Number of tongue flicks
60
50
40
30
20
10
0
Water
Cologne
Earthworm
Slug
Odor

TABLE 1. Significant differences between odor categories (all *P*–values ≤ 0.001; *s* = standard deviation).

Behavior	Category	Rate[1]	Comparison	Q-value
Latency to 1st tongue flick	Water	13.1, *s* = 5.36	Water – Earthworm	7.15
	Cologne	16.7, *s* = 4.69	Water – Slug	9.0
	Earthworm	6.0, *s* = 1.87	Cologne – Earthworm	10.76
	Slug	4.1, *s* = 1.57	Cologne – Slug	12.61
Mean tongue flick	Water	12.0, *s* = 3.22	Water – Slug	27.69
	Cologne	6.2, *s* = 3.0	Cologne – Slug	33.53
	Earthworm	13.8, *s* = 4.09	Earthworm – Slug	25.92
	Slug	39.7, *s* = 15.79		

[1]Rate for Latency to 1st tongue flick in seconds; rate for Mean tongue flick in flicks/1 min.

CHAPTER SIX

EFFECTS OF SHELTER AND PREY ODOR AVAILABILITY ON THE BEHAVIOR OF *DIADOPHIS PUNCTATUS*

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ABSTRACT: We examined the effects of shelter and prey odor on the behavior of the ring-necked snake (*Diadophis punctatus*) over a 23-h period. The prey odors tested were: lizard, snake, mouse, (plus water as control). In experiment one, responses to each odor were tested separately in various shelter and odor combinations. Results showed that snakes preferred shelter to no shelter quadrants, often selecting a quadrant if it also had prey odor in the form of a snake scent, followed by lizard. However, snakes avoided quadrants containing mouse (adult) odor. In experiment two all three odors plus water were presented simultaneously. We found that snakes showed a preference for snake odor over the others, and showed an aversion to mouse odor. Activity in both experiments showed a similar pattern, with activity beginning with lights on, peaking mid-day, thereafter, activity tapered off as snakes began taking up residence in a shelter just before lights off. Prey preferences seen in this study correlate with results of field studies showing a diet comprised mostly of snakes (and some lizards), while activity exhibits strong endogenous diurnal movements.

Key words: Microhabitat; Foraging; Prey choice; Circadian rhythms; Diadophis punctatus

AMONG the factors that have an effect on the behavior of squamates, the presence of and availability of refugia and prey play critical roles. In response to these factors, these reptiles may change behavior within or between season and across various habitats (Beck and Martin, 2003). These responses may also vary ontogenetically (Eskew et al., 2009), and between the sexes (Whitaker and Shine, 2003). Refugia provide more than just

protection against predators (Downes, 1999); reptiles may utilize such sites for thermoregulation (Slip and Shine, 1998), and ambushing prey (Clark, 2004). This is especially true for many species of snakes that are sit-and-wait predators (Theodoratus and Chizar, 2000).

However, most research on microhabitat selection in reptiles has been conducted on primarily diurnal species, such as iguanid (Hertz et. al., 2004) or scincid lizards (Quirt et al., 2006). What is known about shelter use by small, nocturnal squamates is limited to studies on gekkonid lizards (Kearney, 2002) or Australian elapids (Downes 1998; Downes and Shine, 1998). For many species of snakes, both prey availability and type can vary across the landscape, and both may have an effect on behavior (Mushinsky and Hebrard, 1977; Luiselli, 2006; Luiselli et. al., 1998).

The behavior of most dipsadine snakes is poorly known, despite the fact that this is a very species-rich clade of snakes distributed throughout the Western Hemisphere (Zug et al., 2001). Nearly all species of dipsadine snakes are found in the Neotropics of Central and South America. However, some species have distributions that extend into Mexico, the United States and southern Canada. The ring-necked snake (*Diadophis punctatus*), which is a small (usually < 65 cm TL) nearctic species of dipsadine snake, with a trans-continental distribution across the United States, and parts of northern Mexico (Stebbins, 2003). Across most its range *D. punctatus* occurs in a wide range of habitat types, from lowland swamps in the southeastern United States to the prairies of the Midwest, as well as riparian zones in the desert southwest (Ernst and Ernst, 2002).

In the Pacific Northwest *D. punctatus* ranges from western Oregon into north-central Washington, with disjunct populations in southeastern Washington, adjacent Idaho and

southeastern Idaho (Nussbaum et al., 1983). Within this range, *D. punctatus* is most often found in forested regions, comprised of Oregon white oak (*Quercus garryana*) savannah, or Douglas-Fir (*Pseudostuga meinziesii*) and Ponderosa Pine (*Pinus ponderosa*) forests (St. John, 2002). Throughout the range of *D. punctatus* it is considered to be a dietary generalist, feeding on both invertebrates and vertebrates (Blanchard, 1979; Fitch 1975). However, the diet in the Pacific Northwest is more restricted with adult *D. punctatus* feeding primarily on squamates such as small snakes and lizard, and juveniles feeding on earthworms and insect pupae (Weaver and Darda, 2004).

Our experiments focused on microhabitat (shelter) selection in *D. punctatus* as it relates to the presence or absence of potential prey. To conduct our experiments, we tested snakes' responses to shelters in combination with odors of three potential prey items (lizard, snake, mouse), plus a control odor (water). In Experiment one individual odors were presented in four combinations with or without shelters. In Experiment two, we presented snakes simultaneously with all three odors, plus the control, with shelters available at all times. Additionally, we recorded the circadian activity patterns of snakes during both experiments. Our purposes were: 1) to identify effects of shelter and prey odor on microhabitat selection, 2) to determine the relative preference for different prey odors, and 3) to characterize the basic activity pattern of *D. punctatus*.

MATERIALS AND METHODS

We conducted our experiments with 12 adult (6 male and 6 female) *D. punctatus* (288–520 mm snout-vent length). All were collected during 2009 from two sites in

southern Kittitas and western Yakima counties in central Washington State. Snakes were housed individually in 26 x 51 cm glass aquaria, and maintained on 12:12 light cycle year around (lights on at 8:30 h and off at 20:30 h). Temperatures in the rooms housing the snakes and where experiments were performed were held at 25–30 C. Snakes were provided with water *ad libitum*, each snake was alternately fed a variety of prey items (various species of lizards, snakes, and nestling mice) on an irregular basis. To control for bias that may arise from feeding snakes exclusively on one prey species. We maintained snakes under these conditions for at least three weeks before experimental trials were begun.

Prey items used during the trials included the Western Skink (*Plestiodon skiltonianus*), and Terrestrial Gartersnake (*Thamnophis elegans*), both of which are known prey items of *D. punctatus* (Weaver and Darda, 2004). Bedding from adult Swiss-Webster mice (*Mus musculus*) was also used as potential mammalian prey. All prey items (except *M. musculus*) were collected from the same localities as *D. punctatus*. Experiments were conducted using square testing arenas (1.25 m wide x 0.5 m high) constructed from compressed fiberglass panels, resting on a metal platform 20 cm above the floor. Overhead lighting provided 12 h of simulated daylight, while 20-watt red, incandescent bulbs were used during the 12 h of darkness. The floor of the testing arena was covered with plain white butcher paper and divided into four equal quadrants using black tape (Fig. 1). Before each trial a fresh piece of butcher paper covered the arena floor that allowed each marked quadrant to show through. Individual prey odors were presented in covered plastic Petri dishes (diameter = 15 cm), with seven evenly spaced holes (diameter = 1.2 cm) drilled through the top of the dish.

Prey odors were collected by placing one to two specimens of either a lizard or snake into 400 cc of distilled water (Beverlander et al., 2006). Prey items were swirled for about 10 min and then removed. This water was poured into the dish, the bottom of which was lined with filter paper. When soiled bedding from cages containing adult mice was used, enough bedding was added to the dish to cover the bottom (Lee and Waldman, 2002; Slusarczyk and Rygielesky, 2004). Controls during each trial consisted of placing a similar amount of distilled water into a dish, again lined with filter paper. During the trials, shelters were provided that consisted of opaque plastic hide-boxes (10 x 6 x 5 cm). Shelters were provided with and without each odor during Experiment one (Fig. 1). During Experiment two, shelters were present with each of the three potential prey odors, plus the control.

Trials were run for 23 h with one hour for change over (between 16:00 and 17:00 h). Snakes were placed into the center of an arena, and kept under a small plastic cup. This was then lifted at the start of a trial, recording commenced, and all personnel left the room. Behaviors were filmed with Panasonic cameras suspended over each arena and recorded with a Panasonic time-lapse VCR.

We recorded the amount of time snakes spent in each quadrant in minutes. This was recorded once a snake's head entered a quadrant and until its head left a quadrant. These times were recorded and totaled for each quadrant during each hour.

Experiment one: shelter-site and prey-odor selection

In experiment one, each snake was provided with a combination of a single prey odor (lizard, snake, mouse) and control odor (demineralized water), with or without a shelter.

Four combinations were used, one for each of the four quadrants: A: no shelter/prey odor, B: shelter/prey odor, C: no shelter/ no prey odor (water), D: shelter/no prey odor (water) (Fig. 1A). The position of the choices was randomly changed at the beginning of each experimental trial. Additionally, the order of the prey item tested was also randomized for each snake.

Experiment two: prey odor preference

During Experiment two the same three odors were tested simultaneously (lizard, snake, mouse), in combination with a control (water; Fig. 1B). To control for shelter effects in this experiment, a hide-box was placed into each of the four quadrants with the door facing the Petri dish holding the odor. As in Experiment one, the position of the choices was randomly changed at the beginning of each experimental trial, with the order of prey item tested randomized.

For each experiment, shelters and Petri dishes were washed between trials with 70% ethanol, rinsed with demineralized water, and allowed to dry overnight. During the set up of experiments gloves (Microflex, non-sterile latex) were used when handling dishes, shelters, and when changing the paper that covered the bottom of the arena floor. When placing the dishes into the arena we were careful not to cross-contaminate quadrants. One week was allowed to pass between trials of the same snake. Snakes were fed after each trial, confirming hunger.

Statistical analysis

Each snake was run twice, its score averaged, and mean scores compared using a non-parametric test (Kruskal-Wallis, *H*-test). When this test produced statistical significance,

we performed a Tukey Test (*Q*-score) test of multiple pair-wise comparisons to discover which were significantly different from one another.

RESULTS

Experiment one: shelter-site and prey odor selection

After placement into the arena at about 18:00 h, snakes generally spent the first few minutes in the center of the arena before moving toward the edges. Shortly afterward snakes made a few movements around the arena, moving along the walls and across the arena investigating in and around shelters. Individual snakes would crawl near the dishes, usually lingering if a dish contained a prey odor. These behaviors usually lasted for only 10–15 minutes. All snakes had settled into a shelter after 30 minutes of movements and remained in shelters until the following day.

Just after lights on (08:30 h), test snakes emerged from shelters and then moved around the arena. During these movements snakes would move from quadrant to quadrant and in and out of shelters. Snakes ignored (crawling past, not pausing) dishes that contained the control (water). When a snake crawled near a dish that contained either a lizard or snake odor they would pause while moving their heads from side to side across the top of the dish.

Overall, during the 23 h trials (94 total) there was a significant quadrant effect for snake (Kruskal-Wallis, $H = 43.610$; $P < 0.001$), lizard ($H = 31.478$; $P < 0.001$), and mouse ($H = 44.082$; $P < 0.001$). During lizard odor trials, post-hoc, pair-wise multiple comparisons (Tukey test) revealed a significant preference for shelter-odor quadrants (B)

over all other quadrants combinations. However, there was no preference for quadrant A (odor) over quadrant C (no odor-shelter). For snake odor trials, a similar trend was noticed, with snakes spending more total time (in minutes) in a quadrant with both a shelter and snake odor (B). During trials when individuals were presented with the mouse odor, most snakes spent significantly less time in a quadrant containing a mouse odor only (A), mouse odor and shelter (B), or no odor-shelter (C), and spent significantly more time in a quadrant (D) without a shelter or mouse odor (Table 1.)

Experiment two: prey odor preference

As in Experiment one, when placed into the arena, snakes spent a few minutes motionless and then moved about the arena, making a few circuits, and investigating both shelters and dishes. Snakes would pause near a dish containing an odor and then continue moving, ignoring dishes with control odor. All snakes moved into a shelter after 30 minutes and remained there until the following day.

When presented with all three odors simultaneously (lizard, snake, mouse) and control (water), each accompanied by a shelter, snakes showed a preference for the quadrant containing the snake or lizard odor ($H = 43.491$; $P < 0.001$), and spent statistically significant more time in these quadrant over mouse and control (Fig. 3). Post-hoc comparisons showed no difference between quadrants containing snake or lizard odor and mouse or control (Table 2).

Experiment one and two: activity patterns

For each prey type, the trials for *D. punctatus* were combined, and the average number of movements for each hour plotted to show activity patterns. Overall, there was no significant difference ($H = 0.334$, $P = 0.883$) in the average amount of movements made by *D. punctatus* for either Experiment one or two. Average amount movements during trials for each prey odor during experiment one were: lizard (mean movement = 6.46 ± 8.73 SD), snake (7.22 ± 10.21 SD), and mouse (mean = 7.22 ± 10.05 SD). During experiment two when all odors were present, snakes moved an average of 6.92 ± 9.62 SD.

At the onset of Experiment one snakes were initially more active for the first few hrs (16:00–17:00), making 11.08 and 11.9 moves, respectively (Fig. 4A), during the lizard odor trials. For both the snake or mouse odor trials, snakes made similar movements during the two-hour test span, (10.02 and 9.71 times, and 10.2 and 9.8 times during each hour, Fig. 4Band 4BC). For all trials, activity stopped shortly after between 18:00 and 19:00, well before lights out (20:30), and did not begin until the following day between 8:00 and 9:00 hour (Fig. 4).

During Experiment two, again we combined both trials for all snakes and averaged scores each hour, then plotted data to show activity patterns. Similar to results of Experiment one, snakes made several movements during initial introduction, and all snakes settled into a shelter before lights out. Movements plateaued between 19:00 and 21:00 h, with an increase in activity from 12:00 to 13:00 h. Activity steadily increased throughout the day and dropped off equally steadily beginning at 14:00, with all activity stopping around 17:00 (Fig 1. D).

DISCUSSION

Experiment one: shelter and prey odor selection

During Experiment one, *D. punctatus* showed a preference for quadrants with snake or lizard odors presented in combination with a shelter over other combinations without a shelter. Time spent in quadrants with these odors and shelter was significantly greater than those with odor alone. Ring-neck snakes exhibited significantly less interest in a quadrant if mouse odor was present and than if mouse odor was absent (Fig. 2). If a *D. punctatus* approached a quadrant with mouse odor it quickly turned away from the quadrant and moved away in a rapid manner. We interpret these responses to mouse odor, relative to water, as an aversion to, even active avoidance of adult mouse odors. Our general observations, reported above, are also consistent with this interpretation.

A strong selection for quadrants with snake odor (plus shelter), over even a lizard odor is consistent with current field work in Washington state (Weaver and Darda, 2004) as well as western Oregon (O'Donnell et al., 2007).

Experiment two: prey odor preference

During this experiment, *D. punctatus* behaved in much the same way as during Experiment two (all three prey odors plus control presented simultaneously). All individuals made just a few movements after introduction to the test arena and then settled into a selected shelter until the following day (8:30, lights on). *Diadophis punctatus* showed a preference for snake odor over lizard, mouse, and control, spending a significantly greater amount of time in those quadrants containing snake odor (plus

shelter), than mouse or control (water). Similar to Experiment one, snakes in Experiment two displayed avoidance behavior when encountering the mouse odor (with or without shelter).

Overall results from both experiments suggest that snakes are not making random movements. The results consistently show a strong preferenc for the combinations of particular prey odors and shelter, especially snake odor. Little time was spent in quadrants lacking a shelter, with or without odor. Snakes avoided quadrants with mouse odor, and our observations indicate such behavior may be in response to the odor of an adult mouse as a potential threat.

Experiment one and two: activity patterns

We observed no significant difference in the activity patterns of *D. punctatus* during either experiment one or two, but there were some distinctive movements and behaviors displayed by *D. punctatus* during trials. When first placed into the arena, most snakes made just a few movements around the arena, and all snakes moved into a shelter until the following day.

During both Experiments one and two, there was a single peak in activity patterns, which occurred around 12:00. There were no differences in activity patterns between the two experimental conditions. During Experiment one activity peaked during 11:00 and 12:00 h. During Experiment two, activity peaked an hour earlier at 12:00 and 13:00 h. During both experiments, activity began just after lights on (8:30 h) and showed a slow steady decline after 15:00, with all activity stopping at or around 18:00 h.

Avoidance of mouse odor by *D. punctatus* is consistent with similar behavioral work on a small, cryptic dipsadine, the desert nightsnake (*Hypsiglena chlorophaea*). This species also showed an extreme aversion to mouse odor (Weaver and Kardong, 2009). Like *H. chlorophaea*, *D. punctatus* lacks an efficient venom-delivery system possessed by many snakes which feed on rodents (Kardong, 1993). The Duvernoy's secretions and slightly enlarged teeth on the maxilla do not enable *D. punctatus* to capture, subdue and ingest such prey types (Kardong, 1996; 2002).

The diurnal movement patterns by *D. punctatus* during our experiments is in contrast to reports of nocturnal or crepuscular activity (Ernst and Ernst, 2003) in other parts of its range. Movements made by individuals peaked during mid-day, with a sharp decline well before lights out. No snakes displayed movements just before lights off or on (crepuscular movements). This circadian rhythm is consistent with earlier published field work on *D. punctatus* in central Washington State (Weaver and Darda, 2004). Throughout much of central Washington State, *D. punctatus* is sympatric (and syntopic) with *H. chlorophaea*. Both species can be found in rocky areas within oak-woodland and in canyons, prefer rocks as refugia and feed on squamate reptiles. The entirely diurnal movements made by *D. punctatus* is in direct contrast to the entirely nocturnal *H. chlorophaea* (Weaver and Kardong, 2009, Weaver in press). The overlap of distribution and habitat preference may correspond to the preference for snake prey by *D. punctatus*. *Hypsiglena chlorophaea* while feeding on some snakes shows a strong preference for small lizards (Weaver, 2010). Thus, by altering both activity pattern and prey preference, *D. punctatus* may avoid direct competition with *H. chlorophaea*.

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FIG. 1.—Test Arena. (a) Experiment one. For each of the four quadrants A–D, a choice was provided—A: no shelter, prey odor; B: shelter,prey odor; C: shelter,no prey; D: no shelter,no prey odor. (b) Experiment two. An odor was provided in each of the four quadrants A–D—A: Mouse (M), B: Snake (S), C: Lizard (L), D: water, plus a shelter in each quadrant. The four odor/shelter combinations were changed and positioned at random during each of the trials. Circles, petri dishes with prey odor (closed circles) or water (open circles); rectangles, shelters.

FIG. 2.—Total amount of time (minutes) spent in quadrants for all snakes during each 23 h trial for Experiment one (shelter and odor choices).

FIG. 3.—Total amount of time (minutes) spent in quadrants for all snakes during each 23 h trial for Experiment two (prey odor preferences).

FIG. 4. —Activity patterns. Average number of movements for all snakes per hour during the 23 h period. A–C show activity patterns for Experiment one for each of the three prey odors—lizard, snake, mouse. D, shows activity patterns for Experiment two, where all three prey odors and water were presented simultaneously.

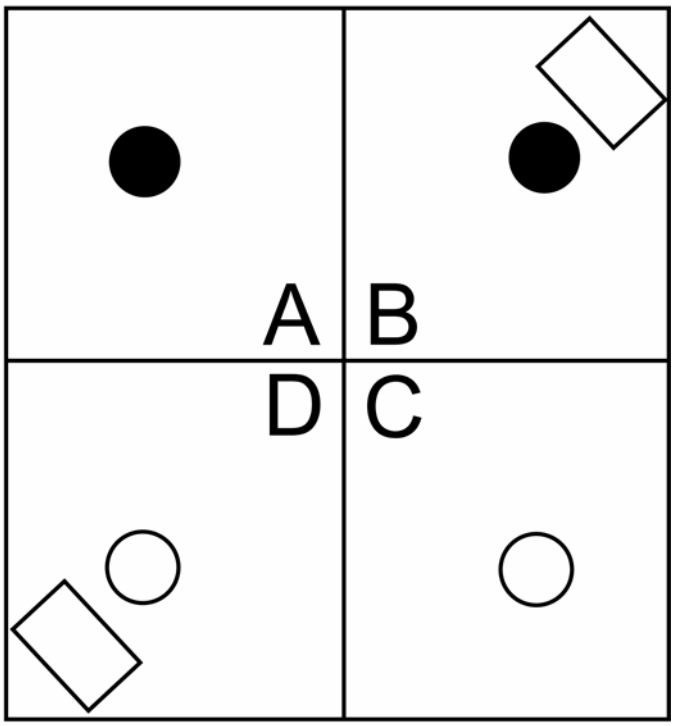
TABLE 1.—Shelter-Site and Prey Odor Selection During 23-h Trials. A: No shelter/Odor; B: Shelter/Odor; C: No shelter/No Odor; D: Shelter/No Odor. *Significant at $\alpha = 0.05$. NS (not significant). Results of pair-wise multiple comparisons (Tukey test) in parentheses.

	Lizard				Snake				Mouse			
	A	B	C	D	A	B	C	D	A	B	C	D
A	—	0.050*	NS	NS	—	NS	NS	0.050*	—	NS	0.050*	0.050*
		(8.226)	(1.516)	(1.320)		(5.347)	(2.894)	(5.856)		(2.969)	(5.938)	(8.908)
B	—	—	0.050*	0.050*	—	—	0.500*	0.050*	—	—	NS	0.050*
			(8.378)	(8.378)			(5.856)	(8.908)			(2.969)	(5.938)
C	—	—	—	NS	—	—	—	NS	—	—	—	NS
				(0.196)				(3.052)				(2.969)

TABLE 2.—Prey Odor Preference During 23-h Trial. *Significant at $\alpha = 0.05$. NS (not significant). Results of pair-wise multiple comparisons (Tukey test) in parentheses

	Lizard	Snake	Mouse	Control
Lizard	—	NS	0.050*	NS
		(3.072)	(5.835)	(2.763)
Snake		—	0.050*	0.050*
			(8.908)	(5.835)
Mouse	—	—	—	NS
				(3.072)

a



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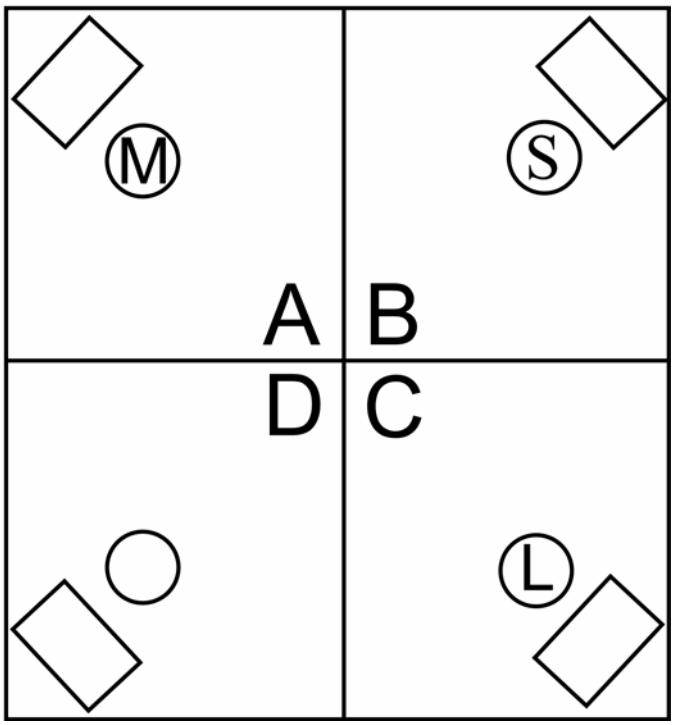

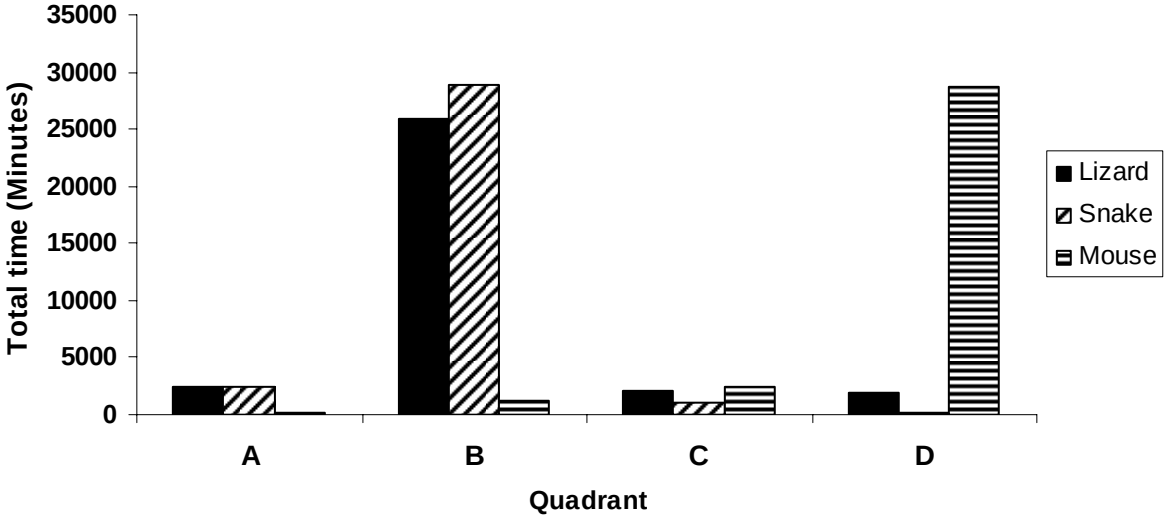
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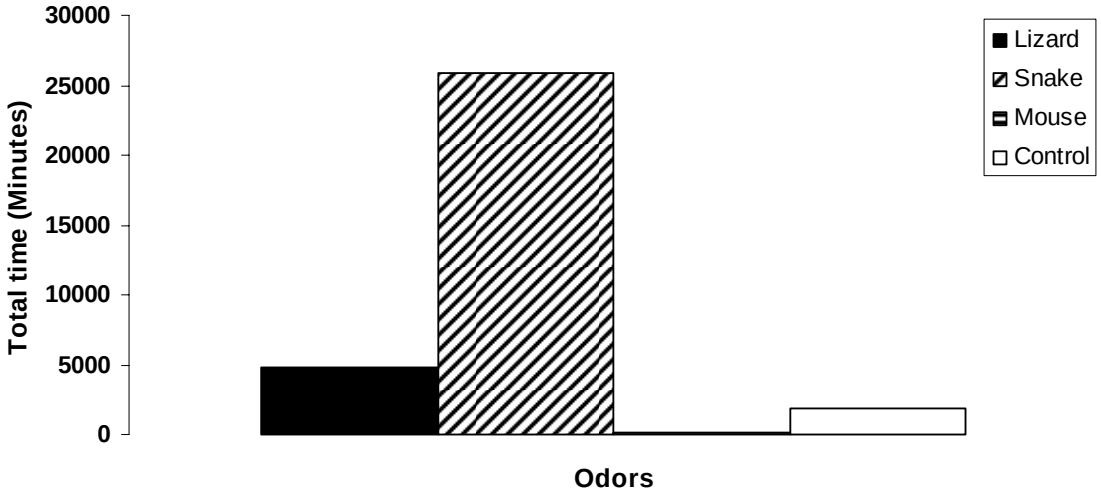
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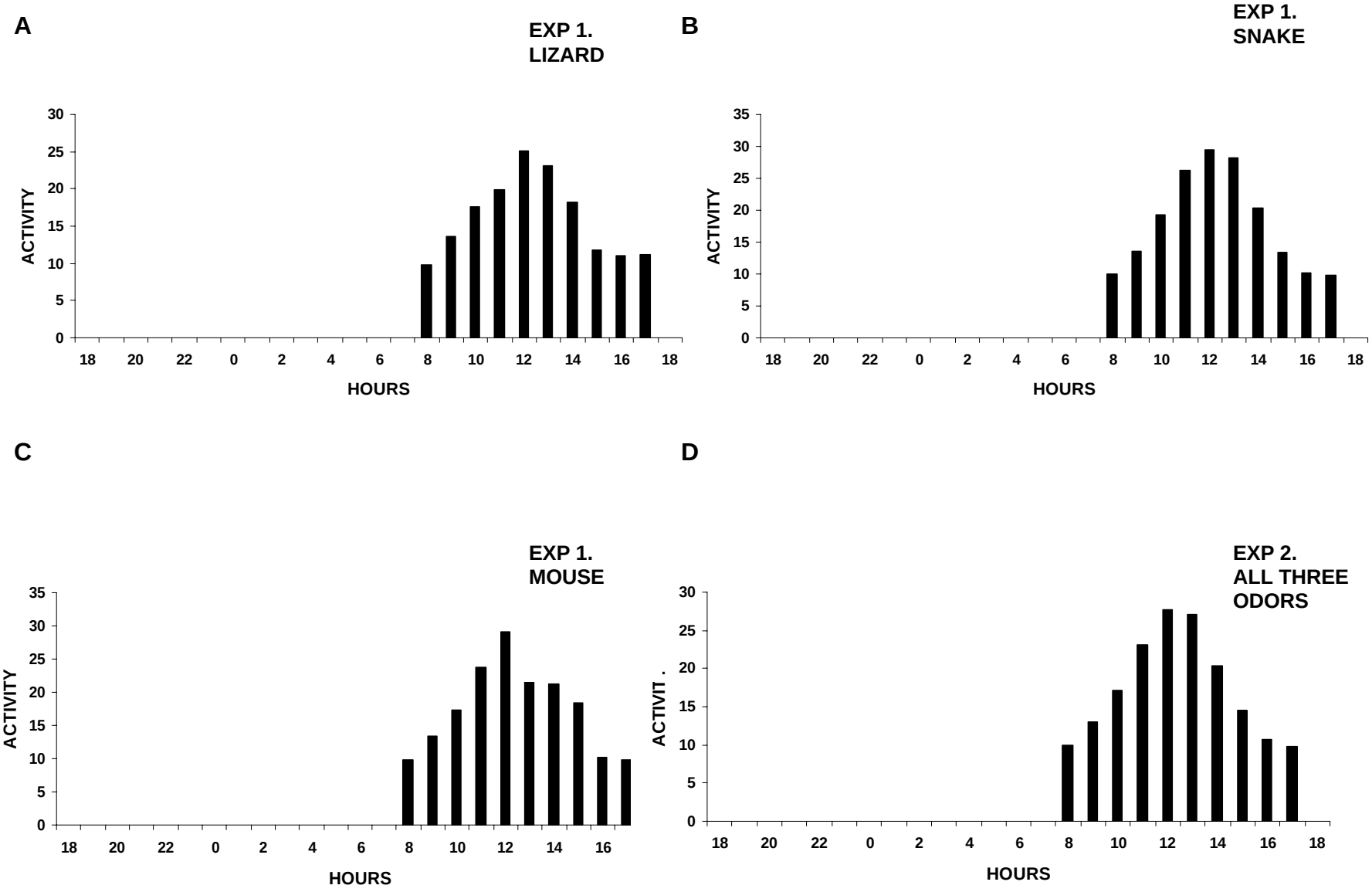
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