Exploring ecological correlates associated with dorsal colour variation in garter snakes.

by

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B.E.S., York University, 1996
M.Sc., University of Victoria, 2003

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in the Department of Biology

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ABSTRACT

Colours influence numerous aspects of an animal's ecology and the adaptive significance of colour variation has been intensively studied in diverse taxonomic groups. This study was motivated by the question: Why do garter snakes vary in colour? To answer this question, I focused on Western Terrestrial Garter Snakes, *Thamnophis elegans*, which exhibit geographic variation in colouration (dark morph vs. light morph), and two different species of garter snake that occur in the same geographic region but vary in colour –light *T. elegans* and the Common Garter Snake, *T. sirtalis*. My work provides an objective quantification and analysis of snake colour and evaluates the influence of colour variation on ecological processes such as thermoregulation, crypsis, and antipredator behaviour.

I compared body temperatures (T_bs) with available thermal opportunities, both in wild snakes and in a field experiment, to determine how snakes with contrasting colours differ in thermoregulation and temperature-dependent behaviours. Gravid females of the light and dark colour morphs of *T. elegans* exhibited comparable thermoregulatory behaviour at high temperatures; however, dark *T. elegans* maintained elevated T_bs when available temperatures dropped. In the field, dark-coloured snakes were more likely to be moving when first detected when T_bs were high, but this trend was reversed in light *T. elegans*.

I quantified crypsis of snakes, in terms of colour and brightness, by measuring the spectral reflectance of snakes and the surrounding habitat. These data were visually modeled from the perspective of potential snake predators and human researchers. Overall, snakes selected basking sites that maximized crypsis and both colour morphs of *T. elegans* were equally cryptic. There was evidence suggesting that *T. sirtalis* was more cryptic than light *T. elegans* to snake predators. I collected a series of behavioural measurements for snakes pre- and post-capture. Light *T. elegans* were more likely to be moving when originally detected in the field than dark snakes. Distance to cover and injuries were important factors in explaining the antipredator behaviour of snakes in the field. Snakes became generally faster with increasing T_bs, but differences attributable to colour morph were not straightforward. A higher proportion of *T. elegans* of both colour morphs exhibited some type of movement when exposed to a simulated predatory attack. *Thamnophis sirtalis*, on the other hand, hung limp and motionless in the air. The less cryptic light *T. elegans* had a higher probability of having an injury than *T. sirtalis* but injury patterns between the equally cryptic light and dark *T. elegans* differed by sex.

The relationships between colour and these various traits were complex, but, taken together, they highlighted how thermal ecology, crypsis, and anti-predator behaviours were related to a snake's visual appearance. These results therefore provide an ecological underpinning for future genetic studies to identify potential candidate genes that may be responsible for the control of colour pattern in garter snakes.

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CHAPTER 1

INTRODUCTION

Phenotypic variation refers to differences in developmental (Thorpe 2006), physiological (Prosser 2008), behavioural (Arnold and Bennett 1998), and morphological (Bjorklund 1990) traits that occur among individuals of the same species. As a result of this variation, individuals incur different fitness-related consequences (e.g. longer life span, faster developmental rate, etc.) on which natural selection will act (Grant et al. 1976, Endler 1980, Dingemanse and Reale 2005). Morphological phenotypic variability, such as variation in colour, has been the focus of many of the early studies of adaptation. Starting with a number of influential publications (Poulton 1890, Cott 1940), the adaptive significance of colour variation has been intensively studied in diverse taxonomic groups such as moths (Kettlewell 1955, 1956), snails (Cain and Sheppard 1950, 1952), fish (Endler 1980, Endler 1987), and birds (Hill and Montgomerie 1994, Hunt et al. 1998).

Colours have been shown to influence numerous aspects of an animal's ecology including the ability to communicate (Endler 1987, Siddiqi et al. 2004), obtain food (Tso et al. 2002), regulate body temperature (Bittner et al. 2002), and avoid predators (Losey et al. 1997). Variation in colour also has important implications for life-history traits such as egg maturation rate (Ellers and Boggs 2004), developmental rate (Corn 1981), reproductive frequency (Roulin et al. 2003), and survivorship probability (Forsman 1995). Often, different suites of traits covary with each other and so identifying relationships with colour is not necessarily straightforward (Endler 1995, Forsman et al. 2002).

Optimal colours may differ for ecological and life-history functions; thus, a particular colour that is adaptive in one context may entail a fitness cost in another. For example, the giant wood spider, *Nephila maculata*, experiences a tradeoff between foraging success and anti-predator behaviour that varies with colour morph (Tso et al. 2002). Such tradeoffs or compromises are common in nature, and sometimes result in colour polymorphisms at the population level (Allen 1988, Tso et al. 2002, Civantos et al. 2004).Whether different phenotypes evolve within a species will also presumably depend on both the differential fitness of alternative phenotypes in different environmental contexts and the relative frequency with which those contexts are encountered (Levins 1968).

My research addresses the evolution of animal colouration. More specifically, I am interested in what, if any, ecological variables are correlated with colour variation in snakes and in the selective advantages and disadvantages that certain colours may confer. Snakes are useful models for testing hypotheses about the evolution of colour morphology in vertebrates because they are widely distributed, ecologically diverse, and exhibit a broad range of colour variation, both phylogenetically and geographically (Jackson et al. 1976).

Colour in snakes appears to serve numerous functions and can vary between species. For example, the colours of some snakes appear to serve an aposematic function, advertising the dangerous and venomous qualities (Brodie and Janzen 1995); these in turn are sometimes mimicked by non-venomous snakes (Savage et al. 1990). Some species change colour (Wilson et al. 2007) or pattern as they grow (Creer 2005). Other species exhibit significant within-population colour variation (Brodie 1989), while others vary geographically (e.g. Western Terrestrial Garter Snakes, *Thamnophis elegans*). This substantial variation provides a tremendous resource for the study of colour and its adaptive significance, but studies so far have been few and have only begun to address this issue.

My study was originally motivated by the question: Why do garter snakes vary in colour? The work presented here focused on Western Terrestrial Garter Snakes, *T. elegans*, that exhibit geographic variation in colouration (dark morph vs. light morph). This intraspecific comparison of colour morphs allowed the assessment of relationships between colour and different ecological parameters within a species. However, because this relationship was potentially confounded by differences in geography, similar comparisons were also included for two different species of garter snake that occur in the same geographic region but vary in colour. This interspecific comparison included the light morph of *T. elegans* and the Common Garter Snake, *T. sirtalis*, which resembles the dark morph of *T. elegans* in colour.

Study site & study species

I undertook field work from 2005-2008 at three study sites in British Columbia (BC), Canada where *T. elegans* and *T. sirtalis* occur (Fig. 1). Dark *T. elegans,* or the dark morph, occurs in coastal BC and I studied it at Cowichan Bay, BC and Rithet's Bog in Victoria, BC (Fig. 1). Cowichan Bay and Rithet's Bog are estuarine and bog habitats respectively and snakes are widely distributed throughout these sites. Snakes are dark (often black) in colour with one dorsal and two lateral stripes, which vary in colour from orange to yellow or white (Fig. 2a).

Figure 1. Map of British Columbia, Canada, illustrating the three study sites where Western Terrestrial Garter Snakes, *Thamnophis elegans*, and Common Garter Snakes, *T. sirtalis*, were studied. The dark morph of *T. elegans* was studied in Cowichan Bay, BC (black star) and Rithet's Bog in Victoria, BC (black triangle). The light morph of *T. elegans* and *T. sirtalis* was studied in the Creston Valley Wildlife Management Area, in Creston, BC (black diamond). Map is courtesy of Weller Cartographic Services Ltd (Nanaimo, BC).

Light *T. elegans*, or the light morph, occurs in the interior of the province and I studied it at the Creston Valley Wildlife Management Area (CVWMA) in Creston, BC (Fig. 1). The CVWMA is a large wetland complex and snakes are broadly distributed throughout this habitat. Light *T. elegans* are light brown in colour with one dorsal and two lateral tan stripes and display a regular pattern of black dorsal and lateral blotching (Fig. 2b). I also studied *T. sirtalis* at the CVWMA. *Thamnophis sirtalis* exhibits similar colouration to the dark morph of *T. elegans* – it is black with prominent dorsal and lateral stripes that are typically yellow-orange; red lateral blotching is often, but not always, present (Fig. 2c).

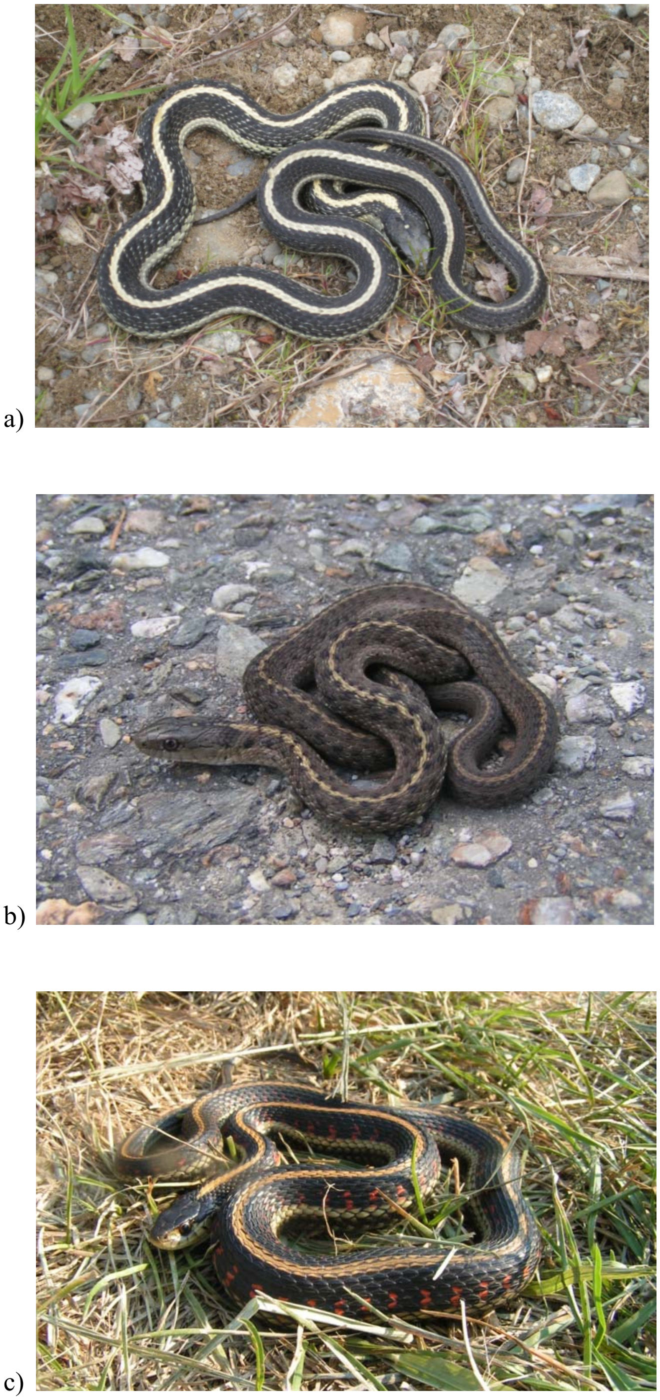

Figure 2. Photographs of the a) dark morph of the Western Terrestrial Garter Snake, *Thamnophis elegans*, b) light morph of *T. elegans*, and c) Common Garter Snake, *T. sirtalis*.

The overall aim of this study was to combine objective methods of colour measurement and visual modeling approaches to investigate the relationships between dorsal colour and a suite of ecological variables in garter snakes. Potential hypotheses explaining the adaptive function of colouration both within and between species centred on three interrelated hypotheses that include thermal advantages, protection from predators, and colour-related patterns of behaviour. More specifically, this research focused on:

1. Colour and temperature:

Temperature is a major physical factor that influences the physiology, behaviour, and general ecology of ectotherms (Stevenson et al. 1985). Because the absorption and reflection of radiant energy is affected by colour, it is a morphological trait that is directly linked with the thermal ecology of snakes (Cooper and Greenberg 1992). For example, melanistic Common Garter Snakes, *Thamnophis sirtalis*, maintain higher mean body temperatures than striped conspecifics when measured in the laboratory and this thermal advantage becomes apparent during the colder parts of the active season (Gibson and Falls 1979). In Chapter 2, I tested relationships between intra- and interspecific variation in dorsal colouration in garter snakes and thermoregulation measured in outdoor enclosures, heating rates recorded in laboratory experiments, and general behavioural traits observed in field captures.

2. Colour and crypsis:

Predation is a major selective force and, as a result, prey organisms often exhibit morphological adaptations to avoid being eaten (Swaddle and Lockwood 1998, Bergstrom and Reimchen 2003). For animals that face threats from visual predators, crypsis, or the visual similarity between an individual and its background, has evolved to help minimize detection (Endler 1978). For example, extensive research in insects has demonstrated how crypsis can contribute to increased survivorship (Kettlewell 1956, Sandoval 1994, Lyytinen et al. 2004). In Chapter 3, I investigated how crypsis, based on colour and brightness, varied between sites where snakes were found basking vs. nearby random sites as well as between habitats of the other colour morph and species. I also evaluated how intra- and interspecific crypsis differed among snake predators, including human researchers.

3. Colour and behaviour:

Crypsis is a relative measure that depends on the background against which an animal is found and immobility forms an integral part of this strategy in some animal groups (Heinen and Hammond 1997, Cooper et al. 2008). Animals that move not only compromise crypsis but can potentially attract predators (Gonka et al. 2000, Hoese et al. 2008, Ryer et al. 2008) and thereby should experience direct fitness-related consequences (Vallin et al. 2006). For example, experimental work on aquatic larval insects has demonstrated that these animals require both crypsis and immobility to avoid being preyed upon by stickleback fish, *Gasterosteus aculeatus* (Ioannou and Krause 2009). In Chapter 4, I examined potential associations between snake colour, behaviours exhibited in the field, performance measured in the laboratory, and injury rates of field captures.

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CHAPTER 2

DOES COLOUR CONFER DIFFERENTIAL THERMAL BENEFITS? INVESTIGATING RELATIONSHIPS BETWEEN DORSAL COLOURATION AND THERMOREGULATION IN GARTER SNAKES.

INTRODUCTION

Temperature is a key abiotic factor that influences numerous aspects of the biology of all organisms. In ectothermic animals, for example, temperature affects the rate of biological processes, such as enzyme and membrane function (Gracey et al. 1996), which, in turn, influences where and when animals can be active (Adolph and Porter 1993, Coxwell and Bock 1995, Hailey and Coulson 1996, Bozinovic and Vasquez 1999, Calosi et al. 2010). Terrestrial ectotherms, such as snakes, depend on external sources of heat from radiation and conduction (Peterson et al. 1993) to maintain body temperatures suitable for a variety of functions, including development (Burger 1998), growth (Autumn and DeNardo 1995), digestion (Stevenson et al. 1985), and locomotory performance (Stevenson et al. 1985, Brodie and Russell 1999, Isaac and Gregory 2005). Because external temperatures usually vary in time and space (Peterson 1987), maintaining optimal body temperatures (T_bs) thereby becomes difficult. High-latitude environments, for example, have been shown to be thermally challenging for snakes because optimal temperatures are restricted to the short activity season (Blouin-Demers and Weatherhead 2001) and, even then, are not constantly available (Peterson 1987, Isaac and Gregory 2004).

In addition to environmental factors, the ability to achieve optimal T_bs depends also on the behaviours and properties of snakes themselves. Snakes use a variety of specific behaviours to manipulate T_bs, including postural adjustments and body orientation (Johnson 1972) as well as habitat selection (Adolph 1990, Blouin-Demers and

Weatherhead 2001). Morphological traits, such as body size, influence the rates of heating and cooling in various snakes (Ayers and Shine 1997). In addition, body colour has also been shown to affect the absorption and reflection of radiant energy (Cooper and Greenberg 1992). Specialized skin cells called melanocytes produce melanin (Bechtel 1978), the colour pigment that can absorb incoming solar radiation, and is often linked to the ability to heat quickly and attain high T_bs (Watt 1968, DeJong et al. 1996, Forsman 1997). As such, body colour has been shown to contribute to the thermal ecology of snakes and has been studied in species where colour variation occurs. For example, the melanistic morph of the European adder, *Vipera berus,* exhibits higher heating rates and maintains slightly higher mean T_bs than the zigzag-patterned conspecific morph (Forsman 1995). In addition, the melanistic morph of the Japanese four-lined snake*,* *Elaphe quadrivirgata*, not only heats faster (Tanaka 2005) but also uses different thermoregulatory strategies to maintain elevated T_bs compared to the striped form (Tanaka 2007). These studies illustrate the connections between skin colouration and T_bs in snakes and highlight the potential differences in thermoregulation approaches that may occur as a result.

If a snake's colouration confers thermoregulatory benefits that are linked with an increase in overall fitness, then, all else being equal, there should be strong selective pressure on colour. Colours that contribute to thermoregulation may, however, have multiple functions that interact with the requirements of other activities or morphological traits (Lindstedt et al. 2008). For example, in melanistic Common Garter Snakes, *Thamnophis sirtalis*, dark snakes maintain higher mean equilibrium T_bs than their striped conspecifics, however, body size mediates this difference (Bittner et al. 2002). Only large melanistic individuals maintain significantly higher T_bs, suggesting that melanism may be more important for large-bodied snakes, such as gravid females, that could potentially benefit from accelerated embryonic development (Bittner et al. 2002). Because colour has the ability to influence thermoregulation as well as other ecological and life-history characteristics (Ahensjo and Forsman 2003), we might therefore expect colour to evolve in concert with other traits, such as locomotory performance or anti-predator behaviours (Forsman et al. 2002).

If dark colours are associated with higher T_bs that confer thermoregulatory benefits and are in fact ‘better’ (Huey and Kingsolver 1989), why then do light-coloured individuals persist? In other words, if selection for high T_bs is strong, and dark colouration is directly linked with high T_bs, we should expect light-coloured individuals to perform worse and hence disappear from populations or species. Lighter or differently patterned morphs may, however, benefit from selection for other advantages that operate either via direct or indirect mechanisms. For example, melanistic *V. berus* and *V. latastei gaditana* experience a higher risk of predation than their zigzag-patterned conspecifics, and are therefore more likely to incur direct fitness costs (Andren and Nilson 1981, Niskanen and Mappes 2005). In other cases, however, the costs associated with colouration may be incurred indirectly – the survival rates of polymorphic pygmy grasshoppers, *Tetrix subulata*, are dependent on jumping ability and reaction distance from predators, which, in turn, are modulated by temperature (Forsman and Appelqvist 1998). That is, the lighter striped morph has enhanced survival at low temperatures when jumping performance is poor and reaction distance between predators is short compared to their darker conspecifics. These kinds of tradeoffs are not necessarily straightforward and have been shown to vary with body size and sex (Forsman et al. 2002, Ahensjo and Forsman 2003); ultimately, however, these tradeoffs can lead to polymorphisms within populations (Andres et al. 2002, Bond 2007).

In general, my research focuses on identifying what, if any, ecological correlates are associated with colour variation in snakes. Here, I investigate potential relationships between body colour and thermal ecology, both within and between two species of garter snakes in British Columbia, Canada. I evaluate the thermal ecology of light- and dark-coloured morphs of the Western Terrestrial Garter Snake, *T. elegans*, in which geographic variation in colouration occurs. I also compare elements of the thermal ecology of two species of garter snake that vary in colour, but occur in the same site –the light-coloured *T.* elegans and the Common Garter Snake, *T. sirtalis*, which resembles the dark-coloured *T. elegans* in colouration. I test relationships between intra- and interspecific variation in dorsal colour and T_b and how this might influence thermoregulation, heating rates, and temperature-related behaviours in the field. More specifically, I ask the following three questions:

1. How does thermoregulation compare between snake colour morphs/species?

I predicted no differences in T_bs between colour morphs and between species in warm conditions because thermoregulatory opportunities are readily available. In cool conditions, however, I predicted higher T_bs in the dark morph and in *T. sirtalis* because this has been shown in melanistic snakes (Gibson and Falls 1979). Finally, I predicted smaller differences in T_bs between colour morphs and between species for gravid females because of the priority they place on thermoregulation versus other behaviours (Gregory et al. 1999, Blouin-Demers and Weatherhead 2001, Lourdais et al. 2004).

2. Are there differences in heating rates between snake colour morphs/species?

I predicted that dark *T. elegans* and *T. sirtalis* would heat faster than light *T. elegans* when tested in the laboratory as has been shown in other dark-coloured snakes (Tanaka 2005). Body size has also been demonstrated to influence heating rates (Ayers and Shine 1997, Pereboom and Biesmeijer 2003) and I therefore predicted that smaller snakes would heat faster than larger snakes of the same colour.

3. Is there a relationship between T_b, body colour, and various snake behaviours?

I predicted warm snakes would be more likely to be found further away from shelter because they can rely on increased speed to escape capture whereas cold snakes, by contrast, are slower and would be more likely to remain near shelter when basking. Whether cold snakes would be more likely to risk being visible and basking in the open than warm snakes is an open question, however. Similarly, whether cold or warm snakes would be more likely to move when approached is difficult to predict – cold snakes are slower and perhaps should move sooner, but by doing so, they expose themselves to a predator that might not have seen them. I also predicted that the relationship between colour and T_b (questions #1 & #2) would affect behaviours observed in the field; however, these relationships are complex (Forsman et al. 2002) and are therefore difficult to predict.

METHODS

Study area, snake capture & measurements

I undertook field work from 2005-2008 at three study sites in British Columbia (BC), Canada where the light and dark morph of the Western Terrestrial Garter Snake, *Thamnophis elegans,* and the Common Garter Snake, *T. sirtalis*, occur (see Chapter 1, Fig. 1). I searched for snakes on foot and captured them by hand. I measured combined head and body length (snout-vent length, SVL) and weight, determined the sex of each snake, and assessed each female's reproductive condition by gently palpating its abdomen to determine whether it was gravid. I individually marked each snake for future identification by clipping a unique combination of subcaudal scutes on the underside of the tail to ensure that each individual snake was included only once in statistical analyses.

During field work sessions, I noted various behaviours, including distance to shelter, whether or not snakes were moving when first detected, and whether or not snakes that were initially detected when motionless, moved as I approached them. For all field captures, I measured body (cloacal) temperature (T_b) immediately after capture using a quick-reading digital thermometer (HI 9063 thermometer, Hanna Instruments, Singapore). I held snakes by the midsection and the tail in order to minimize temperature change attributable to my handling. I also placed a mercury thermometer on the ground at each capture site to measure potential T_bs that could be attained by snakes, also referred to as operative temperature (Bakken 1992). The thermometer was positioned at the capture site for at least 10 minutes to allow equilibration before reading the temperature. In addition to snakes that were captured in the field, I also recorded T_e for snakes that I did not catch. These data allowed me to estimate the potential T_b of snakes that evaded capture and to indirectly evaluate the relationship between T_b and the probability of evading capture by 'human predators'.

Thermoregulation: enclosure

In 2006, I measured T_bs of light and dark *T. elegans* when they were allowed to choose freely between microhabitats in a series of outdoor semi-natural enclosures. At the time this experiment was conducted, research was focused only on the intraspecific comparison of thermoregulatory abilities in the two colour morphs of *T. elegans.* As a result, *T. sirtalis* were not included in this experiment.

Three identical enclosures were constructed both in Victoria, BC, where dark *T. elegans* occur, and in the Creston Valley Wildlife Management Area, where light *T. elegans* occur. Each enclosure was 5m X 5m and contained cover objects, rock piles, tunnels, basking sites with shelter nearby, and ponds stocked with fish which gave snakes free access to water and food (Fig. 1). The enclosures were covered with netting to deter potential avian predators.

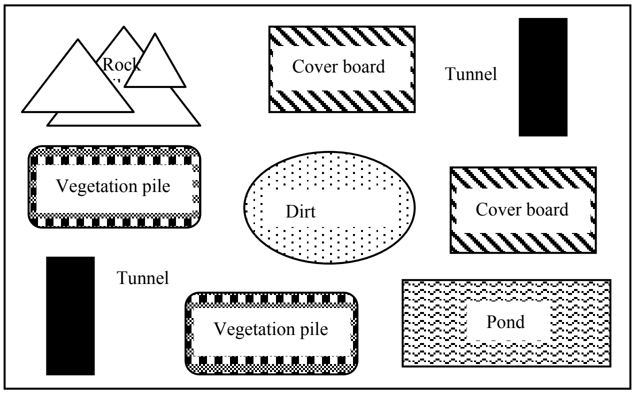

Figure 1. Diagram illustrating the outdoor enclosure used to maintain light and dark Western Terrestrial Garter Snakes, *Thamnophis elegans* for thermoregulation studies. Snakes were provided a variety of cover types including rock piles, cover boards, vegetation piles, pond, tunnels, and a dirt mound.

Operative temperature (T_e) describes the thermal conditions experienced by a snake in a microhabitat when it is not regulating its T_b (Bakken 1992). In other words, T_e

represents the maximum temperatures available to a snake and can be estimated using various types of physical models (Vitt and Sartorius 1999) that share a physical resemblance and measure T_bs closely to those maintained by real snakes (Peterson 1987). I monitored these available temperatures using copper pipe models in one of the outdoor enclosures. Each model consisted of a 700mm long piece of hollow copper tubing, 15mm in diameter and painted to match the two colour morphs of *T. elegans*. Because I was limited in the number of copper pipe models available, I also recorded T_e with StowAway® TidbiT® Temp Loggers (Onset Computer Corporation, Massachusetts, U.S.A.) and compared how closely the two types of models (copper pipe models vs. TidbiT®s) recorded T_e. There was a strong positive correlation between the copper pipe models and TidbiT® dataloggers (dark model: $r^2 = 0.966$, $p < 0.0001$; light model: $r^2 = 0.974$, $p < 0.0001$) and so values recorded by the data loggers were used for all analyses. T_e was measured every 30 minutes to correspond with the frequency of T_b measurements (see below).

Temperature-sensitive radio-transmitters (5.2g SB-2T transmitters; Holohil Ltd., ON) were calibrated in the laboratory and then implanted into 18 large-bodied gravid *T. elegans* (nine of each colour morph) by a veterinarian. Only gravid females were used for this study to minimize potentially confounding effects of sex and reproductive state. Dark *T. elegans* used in this experiment were significantly longer (mean SVL ± SD: 554.1 ± 56.4 mm) than light *T. elegans* (510.1mm ± 26.3 mm; $t_{11.324} = 2.12$, $p = 0.0567$), despite attempts to find similarly-sized snakes.

Following recovery from surgery, six snakes (three of each colour morph) were placed in each of the outdoor enclosures. The unique pulse rate of each radio-transmitter was remotely recorded using a datalogger (SRX 400A, Lotek Wireless Inc., 2006) and later converted to T_b. Body temperature was measured every 30 minutes and treated as an independent measure.

The experiment occurred over a 14-day sampling period in the Creston enclosures. After this time, snakes were moved to the Victoria enclosures and the experiment was continued for another two weeks - snakes gave birth during this time. Because I located and observed snakes each day (see Behaviour section below), I was able to determine the exact day when females gave birth. Following the 2-week testing period in Victoria, the same snakes, now nongravid, were moved back and kept in the Creston enclosures. Following this, they were taken back to the Victoria enclosures for the final 2-week period. The original intention of conducting this experiment in the two locations was to account for the potential influence of site on thermoregulatory behaviour. But, location was unavoidably confounded with time as well as with changes in developmental state and so location was not included as a factor in the analysis. Uneven sample sizes are because of poor transmitter signal reception, snake escapes from their enclosures (two light *T. elegans* and three dark *T. elegans*), or, snake death (one of each colour morph due to unknown causes), which occurred towards the end of the experiment when snakes were nongravid.

Behaviour

Throughout the duration of the enclosure experiment described above, I observed the behaviour of the 18 *T. elegans* at various time periods. I painted identification stripes on each snake to facilitate these observations. Each day was divided into three 4-hour time periods: 7am-11am, 11am-3pm, and 3pm-7pm. I randomly chose one observation period per day and ensured that equal numbers of each time period were sampled throughout the duration of the entire experiment. During this four-hour period, I recorded behaviour once every hour – in total, five observations were collected per snake per time period. The observations were spaced at 1-hour increments so that snakes could move freely between these times. For each snake, I noted if it was visible or hidden and estimated its proximity to the nearest cover object according to the following categories: 1cm (snake immediately beside or on top of cover), 15cm, 30cm, 45cm, & 60cm. To eliminate potential confounding effects of enclosure design on snake behaviour, I excluded observations of snakes that were found immediately next to the enclosure edge. Body temperatures were recorded during this time allowing for comparisons to be made with visibility in the enclosure (vs. not visible) and distance to refuge.

Heating rates

I measured the heating rates of nine snakes – three each of light *T. elegans*, dark *T. elegans*, and *T. sirtalis*, caught during the 2008 field season. Only males were used in this experiment in order to minimize the potential confounding effects of sex. Each snake was tested in a small experimental arena that was heated to 40ºC with an overhead lamp. Snakes were maintained in a refrigeration unit in individual plastic containers until they reached a T_b of 10ºC. Once snakes reached this T_b, they were removed one at a time from the refrigeration unit and placed in the heating chamber where they were allowed to move freely. Every 30 seconds, I picked up the snake and held it by the midsection and tail and recorded body (cloacal) temperature using a quick-read digital thermometer (HI 9063 thermometer, Hanna Instruments, Singapore) and then returned it to the experimental arena. Each trial continued until the snake reached a final T_b of 35ºC. Following each trial, the snake was placed back in its individual container and allowed to rest at least 2 hours until the experiment was repeated. In total, three heating trials per snake were completed.

ANALYSIS

Thermoregulation

I regressed T_b on T_e separately for light and dark *T. elegans* to evaluate thermoregulatory behaviour both in the enclosure and in the field. I performed a similar regression of T_b on T_e for *T. sirtalis* to evaluate thermoregulation in the field only. A slope of 1 in this regression indicates thermoconformity ($T_b = T_e$), whereas a slope significantly less than 1 indicates some level of thermoregulation (independence of T_b from T_e). A slope of 0 represents complete independence of T_b and T_e and indicates thermoregulation. Because the expected relationship between T_b and T_e is nonlinear (i.e. T_bs are expected to level off at a preferred or selected level as operative temperatures rise), I used a modified regression approach, called piecewise regression (Neter et al. 1983). This analysis determines the T_e at which the slope of the T_b vs. T_e regression changes (Isaac and Gregory 2004) – this T_e is hereafter referred to as the 'changepoint T_e'.

I evaluated whether the changepoint T_e differed between reproductive states and colour morphs. Following this, the 'segments' or 'pieces' above and below the changepoint T_e were analysed separately and I compared mean T_bs of each piece using 1-way ANOVA. I then tested the strength of the relationship of T_b vs. T_e for each piece against a slope of 1 and 0 using regression analyses. Body temperatures recorded in the enclosure were measured repeatedly on the same individuals, so to account for these repeated measurements, I nested individuals within colour morph for all analyses.

Heating rates

I calculated thermal time constants following the description outlined in Dzialowski and O'Connor (2001). This approach involves subtracting the temperature of the environmental test chamber (40ºC, in this case) from each body temperature measured throughout the experiment as the animal warms (Dzialowski and O'Connor 2001). The natural log of each difference was calculated and then regressed against time. The slope of the regression line is inversely proportional to the thermal time constant, τ, which is a measure of heating rate. I used ANCOVA to analyse potential relationships between τ (dependent variable) and colour morph (independent variable) and included body weight as a covariate because it affects heating rates (Bittner et al. 2002). Snakes were nested within each colour morph because each individual was tested multiple times. Heterogeneity of slopes was tested by initially including the interaction between weight and colour morph in all analyses, and then dropping it if it was not significant.

Behaviour

I used logistic regression to test the relationship between T_b and the probability of snakes being visible in the enclosure for light and dark *T. elegans*. I then compared T_bs among the five distance-to-cover categories using ANOVA for light and dark *T. elegans*. Because females were gravid when the enclosure experiment began and gave birth partway through the experiment, I included reproductive state (i.e. gravid vs. nongravid females) as a potential factor in all analyses. In addition, observations were repeated for the same 18 snakes, so I nested individuals within colour morph to take this into account.

For field captures, I used logistic regression to test the relationship between T_b and the probability that snakes were moving when first detected. Of those snakes that were observed not moving when first detected in the field, some snakes continued to remain motionless as I approached to capture them whereas others immediately fled. I then used a separate logistic regression to test the relationship between T_b and the probability that these initially immobile snakes moved before capture. Numerous functions are linked with body size in snakes so SVL (mm) was included in these analyses (Stevenson 1985, Finkler and Claussen 1999). These tests also included colour morph/species as a factor to measure the potential influence of colour in these T_b/behaviour relationships. And finally, I used logistic regression to test the relationship between the probability of a snake either being caught or missed vs. T_e for light and dark *T. elegans* and *T. sirtalis*. In this test, T_e was used as a best estimate for potential T_b because actual T_b could not be measured for snakes that got away.

All statistical tests were performed using SAS 9.2. Post-hoc model comparisons were performed using the Tukey-Kramer method. I used Type III sums of squares in all tests of significance and I used the RANDOM/TEST option in PROC GLM of SAS to identify the appropriate F-tests where factors were identified as random (e.g. individual snakes) rather than fixed. If significant effects of treatments were found, I used least-squares means (LSMEANS) to compare means from each treatment and values are reported with mean ± SD. I considered differences to be nominally significant at the 0.05 level. All animal maintenance and experimental procedures complied with guidelines for live reptiles and were approved by the University of Victoria Animal Care Committee.

RESULTS

Thermoregulation: enclosure

Snakes were observed feeding, drinking and using all microhabitat types within the enclosures. Eight of the nine light Western Terrestrial Garter Snakes, *Thamnophis elegans,* gave birth between July 23 and August 4, 2006 and the last of the light *T. elegans* gave birth on August 10, 2006. The first of the nine dark *T. elegans* gave birth on July 30, 2006 and the remaining eight snakes gave birth between August 7 and August 18, 2006.

In total, I collected over 37,000 body temperatures (T_bs) ranging from 6.19ºC to 43.72ºC from the 18 snakes in the outdoor enclosures. Daily T_bs showed a typical pattern that was consistent for gravid and nongravid females (Fig. 2 & 3). Snakes experienced a rapid heating phase in the morning (8am to 10am) followed by sustained high T_bs during most of the day. Snakes then began a cooling phase in the early-evening (6pm) followed by lower T_bs during the night when ambient temperatures fell and refuge was taken.

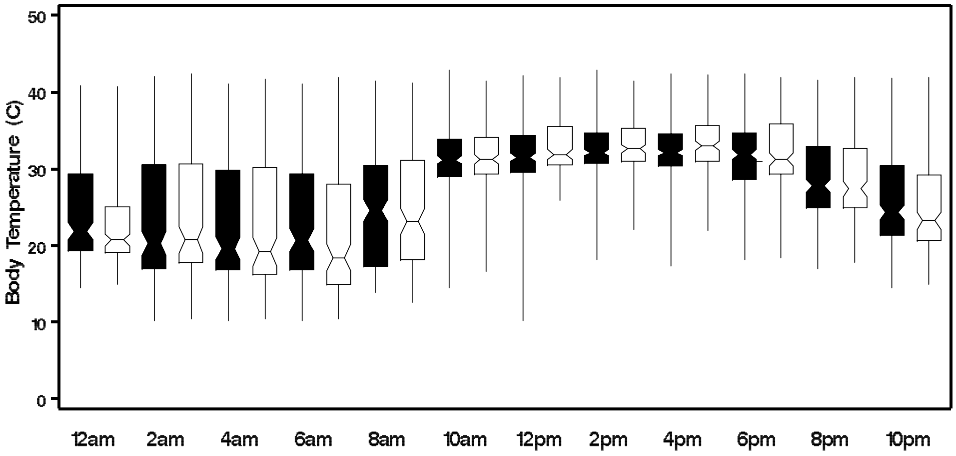

Figure 2. Profile of body temperatures (T_bs) for dark (black boxes) and light (white boxes) gravid Western Terrestrial Garter Snakes*, Thamnophis elegans,* over time. To illustrate the general trend, only T_bs for 12 times are shown. Overall, mean T_bs increased during the day. Variability in T_bs was lowest at mid-day (i.e. 2pm to 4pm) and highest in the early morning (i.e. 2am to 6am). Each box represents 50% of the data. The median is indicated by the notches and lines extend to the minimum and maximum values.

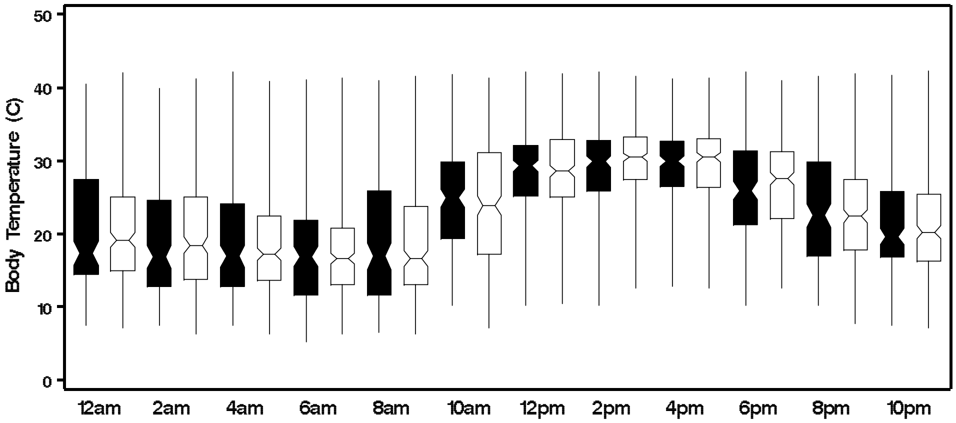

Figure 3. Profile of body temperatures (T_bs) for dark (black boxes) and light (white boxes) nongravid Western Terrestrial Garter Snakes, *Thamnophis elegans,* over 2-hour time periods. To illustrate the general trend, only T_bs for 12 times are shown. Overall, mean T_bs increased during the day and variability in T_bs was generally greater (than gravid snakes) while snakes were nongravid. Each box represents 50% of the data. The median is indicated by the notches and lines extend to the minimum and maximum values.

Overall, gravid snakes maintained significantly higher mean T_bs than nongravid snakes ($F_{1, 1707} = 294.54$, $p < 0.0001$) and this difference was consistent between colour morphs ($F_{1, 1707} = 1.34$, $p = 0.2472$; Fig. 4). The variability of T_bs, as measured by standard deviation, was significantly higher when snakes were nongravid ($F_{1, 1664} = 195.66$, < 0.0001), which was also consistent between light and dark *T. elegans* ($F_{1, 1664} = 1.23$, $p = 0.2679$; Fig. 5).

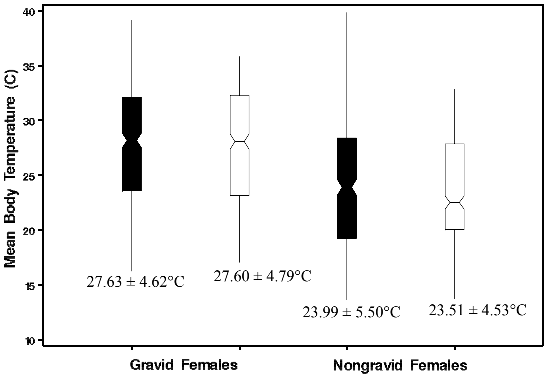

Figure 4. Summary of mean body temperatures (T_bs) measured from dark (black boxes) and light (white boxes) Western Terrestrial Garter Snakes, *Thamnophis elegans*, in outdoor enclosures. Mean values were calculated for each 30-min interval for each snake, when gravid and nongravid. The mean and range of T_bs was comparable between the two colour morphs when gravid and nongravid. Each box represents 50% of the data. The median is indicated by the notches and lines extend to the minimum and maximum values.

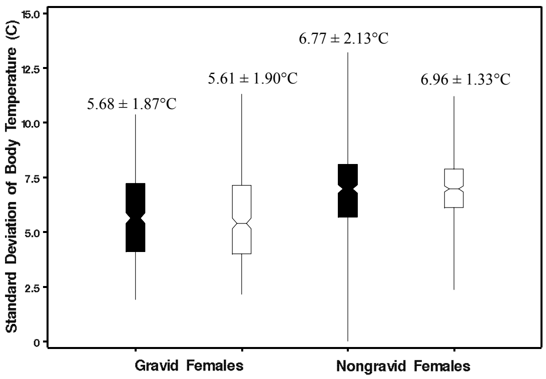

Figure 5. Summary of variability, as measured by standard deviation, of body temperatures (T_bs) for dark (black boxes) and light (white boxes) Western Terrestrial Garter Snakes, *Thamnophis elegans*, in the outdoor enclosure. Variability in T_bs was comparable between the two colour morphs in both reproductive states. Variability in T_bs increased when snakes became nongravid. Each box represents 50% of the data. The median is indicated by the notches and lines extend to the minimum and maximum values.

I used all datapoints to determine the T_e at which gravid and nongravid females began thermoregulating, that is, the changepoint T_e. I was unable to meet the convergence criterion assumption of piecewise nonlinear regression for nongravid females and so they were excluded from this part of the analysis. I first compared the changepoint T_e of gravid snakes for each colour morph in the three enclosures. The confidence limits associated with these changepoint T_es overlapped and so enclosures were combined and the analyses repeated for light and dark *T. elegans*.

The changepoint T_es were similar between light and dark *T. elegans* (29.35ºC vs. 29.09ºC) and so the data were further condensed by combining colour morphs. The analysis was then finally repeated for all gravid females and yielded a changepoint T_e of 29.15ºC. The changepoint T_e corresponded to a T_b of 32.35°C (Fig. 6).

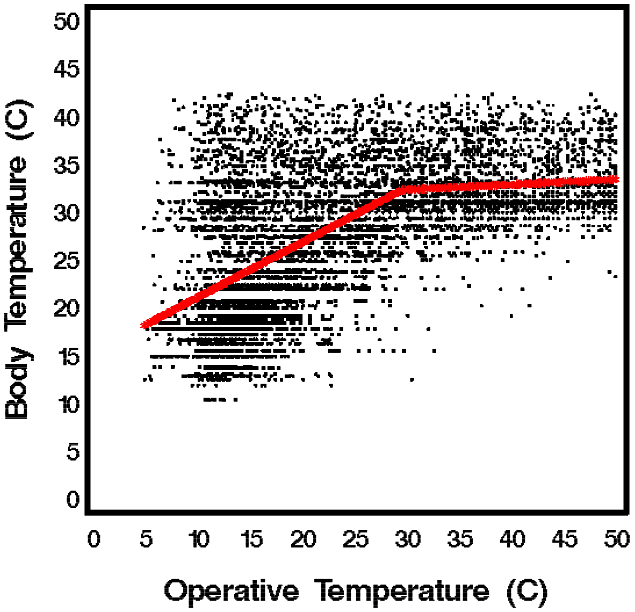

Figure 6. Scatterplot showing body temperature (T_b) vs. operative temperature (T_e) for a gravid Western Terrestrial Garter Snake, *Thamnophis elegans,* in the outdoor enclosure. T_bs for one light *T. elegans* are shown but the same pattern applies for the remaining light *T. elegans* and all dark *T. elegans*. Each dot represents one T_b measured throughout the experiment over all time periods. The red line is the predicted line as determined from the regression. The slope of the regression changed at T_e=29.15°C, which corresponded to a T_b=32.35°C.

I then reduced the dataset to mean values by time for each gravid snake when comparing differences in T_bs above and below the changepoint T_e. That is, I condensed T_bs to mean T_b for each 30-minute sampling period for every snake over all days when gravid. Below the changepoint T_e, dark *T. elegans* maintained significantly higher mean T_bs than light *T. elegans* (25. 27°C vs. 24.72°C; Table 1). Data were divided into two groups, i.e. below and above the changepoint T_e, and analysed separately. For dark *T. elegans*, the slope of the lower piece (0.52) was significantly different from a slope=0 (r^2 = 0.506, $p < 0.0001$) and from a slope=1 ($p < 0.0001$) indicating that snakes were thermoregulating. For light *T. elegans*, the slope of the lower piece was closer to 1 (0.62), but was still significantly different from a slope=0 (r^2 = 0.651, $p < 0.0001$) and from a slope=1 ($p < 0.0001$) suggesting that snakes were also regulating their T_bs.

Above the changepoint T_e, T_bs were similar between the two morphs (32.32 ºC vs. 32.41 ºC for dark and light *T. elegans*; Table 1). The slope of this upper piece was closer to zero (0.07 for dark *T. elegans* and 0.09 for light *T. elegans*), but it still differed significantly from a slope=0 ($p < 0.0001$) and from a slope=1 ($p < 0.0001$) for both colour morphs. But, the ability of the model to account for the variability in the relationship was much lower ($r^2 = 0.100$ for dark *T. elegans* and $r^2 = 0.124$ for light *T. elegans*). I predicted T_bs would level off at these higher T_es and the slope of this upper piece would not differ from 0 but this was not the case.

Table 1. Summary of ANOVA analyses comparing mean body temperatures (T_bs) of light and dark gravid Western Terrestrial Garter Snakes, *Thamnophis elegans*, in the enclosure. Analyses were separated into two groups – T_bs that fell below and above the changepoint operative temperature (changepoint T_e) as determined by nonlinear piecewise regression. Mean T_bs were significantly greater for dark *T. elegans* below the changepoint T_e, however, these differences disappeared above the changepoint T_e.

Comparison with changepoint T_e	**Factor**	**F-value, df, p-value**
Below 29.15ºC	Morph	$F_{1, 536} = 10.28$, $p = 0.0014$
	Individual (morph)	$F_{16, 536} = 13.68$, $p < 0.0001$
	T_e	$F_{1, 536} = 1017.75$, $p < 0.0001$
	Morph * T_e	$F_{1, 536} = 5.72$, $p = 0.0171$
Above 29.15ºC	Morph	$F_{1, 287} = 2.99$, $p = 0.0847$
	Individual (morph)	$F_{16, 287} = 9.34$, $p < 0.0001$
	T_e	$F_{1, 287} = 72.79$, $p < 0.0001$
	Morph * T_e	$F_{1, 287} = 3.37$, $p = 0.0676$

Thermoregulation: field

I collected a total of 337 T_bs from male, nongravid, and gravid light and dark *T. elegans* and *T. sirtalis* in the field. Snakes were active over a range of T_es extending from 6°C to 45°C (Fig. 7) and mean T_bs ranged from 24.94°C for male light *T. elegans* to 28.11°C for gravid light *T. elegans* (Fig. 8).

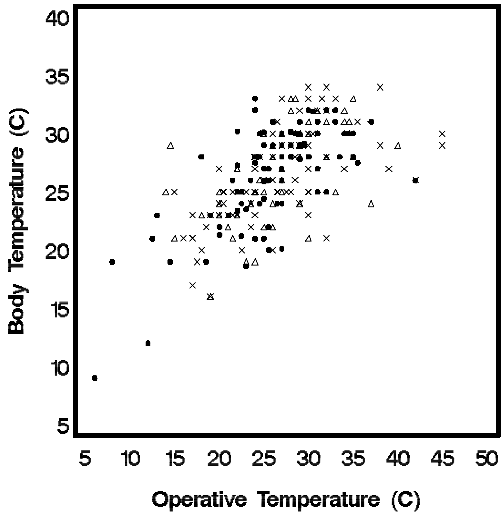

Figure 7. Summary of body temperatures (T_bs) and the associated operative temperatures (T_es) for snakes caught in the field. Light Western Terrestrial Garter Snakes, *Thamnophis elegans* are represented by (X), dark *T. elegans* by (●), and Common Garter Snakes, *T. sirtalis*, by (Δ).

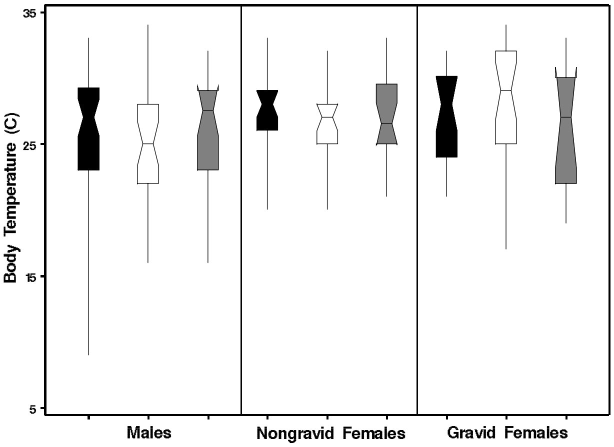

Figure 8. Summary of body temperatures (T_bs) measured in the field for male, nongravid, and gravid female Western Terrestrial Garter Snakes, *Thamnophis elegans*, of both colour morphs and Common Garter Snakes, *T. sirtalis*. T_bs were generally similar within and between each group. Dark *T. elegans* are indicated by black boxes, light *T. elegans* by white boxes and *T. sirtalis* by grey boxes. Each box represents 50% of the data. The median is indicated by the notches and lines extend to the minimum and maximum values.

The convergence criterion assumption associated with nonlinear regression was not met when field T_bs were evaluated for light and dark *T. elegans* and *T. sirtalis*. I therefore applied the changepoint T_e as determined in the enclosure experiment (29.15°C), to gravid light and dark *T. elegans* and used this value as a best estimate for gravid *T. sirtalis*. Work by Charland (1995) demonstrated strong similarities in thermoregulatory behaviour between light *T. elegans* and *T. sirtalis* in Creston and so the application of the changepoint T_e to this group is considered appropriate. All males and nongravid females were excluded from this analysis either because of small sample sizes or because an appropriate estimate of changepoint T_e was lacking.

Mean T_bs of dark and light *T. elegans* and *T. sirtalis* were similar both below (25.64ºC, 26.03ºC and 24.87ºC) and above (31.16ºC, 30.17ºC and 30.57ºC) the changepoint T_e for gravid snakes (Table 2). Below the changepoint T_e, the slope of the regression of T_b vs. T_e (0.547) differed significantly from a slope=0 (r^2= 0.4475, p = 0.0046) and from a slope=1 (p = 0.0144) for dark *T. elegans*. Light *T. elegans* thermoconformed at these low T_es, as the slope (0.689) differed from 0 (r^2= 0.4203, p = 0.0165) but not from 1 (p = 0.2285). For *T. sirtalis*, on the other hand, the slope of T_b vs. T_e (0.911) did not significantly differ from slope=0 (r^2= 0.2330, p = 0.1882) nor from slope=1 (p = 0.8912), owing to the fact that this sample was small and variable.

Above the changepoint T_e, the slope of the regression of T_b vs. T_e (-0.270) did not differ from a slope=0 (r^2= 0.270, p = 0.369) but did differ significantly from a slope=1 (p = 0.0158) for dark *T. elegans*. For light *T. elegans*, the slope of the regression of T_b vs. T_e (0.343) did not differ from a slope=0 (r^2= 0.081, p = 0.346) nor from a slope=1 (p =

0.086). There were insufficient data above the changepoint T_e to evaluate thermoregulatory behaviour of *T. sirtalis.*

Table 2. Summary of ANOVA analyses comparing field body temperatures (T_bs) for gravid females of light and dark Western Terrestrial Garter Snakes, *Thamnophis elegans,* and Common Garter Snakes, *T. sirtalis*. Analyses were divided into two groups - above and below the changepoint operative temperature (changepoint T_e; i.e. 29.15 ºC) as determined in the enclosure experiment. Mean T_bs were similar between colour morphs and between species both above and below the changepoint T_e. All non-significant interactions were removed from the analysis.

Comparison with changepoint T_e	**Factor**	**F-value, df, p-value**
Below 29.15ºC	Colour morph	$F_{1, 26}$= 0.13, p = 0.7194
	T_e	$F_{1, 26}$= 19.40, p = 0.0002
Above 29.15ºC	Colour morph	$F_{1, 15}$= 0.22, p = 0.5550
	T_e	$F_{1,15}$ = 0.86, p = 0.3691
Below 29.15ºC	Species	$F_{1, 19}$= 1.05, p = 0.3184
	T_e	$F_{1, 26}$= 9.36, p = 0.0064
Above 29.15ºC	Species	$F_{1, 1}$= 0.01, p = 0.9349
	T_e	$F_{1, 11}$= 0.97, p = 0.3459

Heating rates

There was no significant interaction between weight and colour morph in either the intra- (p = 0.1366) or interspecific (p = 0.3188) comparisons, so these terms were dropped from further analyses. Overall, light *T. elegans* heated up more rapidly than dark *T. elegans* (τ=3.05 vs. τ=6.67), although this difference was not significant ($F_{1,13}$=1.26, *p* = 0.2817). Light *T. elegans* exhibited approximately the same heating rates as *T. sirtalis* (τ=4.17 vs. τ=4.72; $F_{1,13}$=0.10, p = 0.7586). Smaller-bodied snakes heated more quickly than larger-bodied snakes, but the differences were not significant for either the intraspecific ($F_{1,13}$=1.80, p = 0.2024) or the interspecific comparison ($F_{1,13}$=2.18, p = 0.1634; Fig. 9).

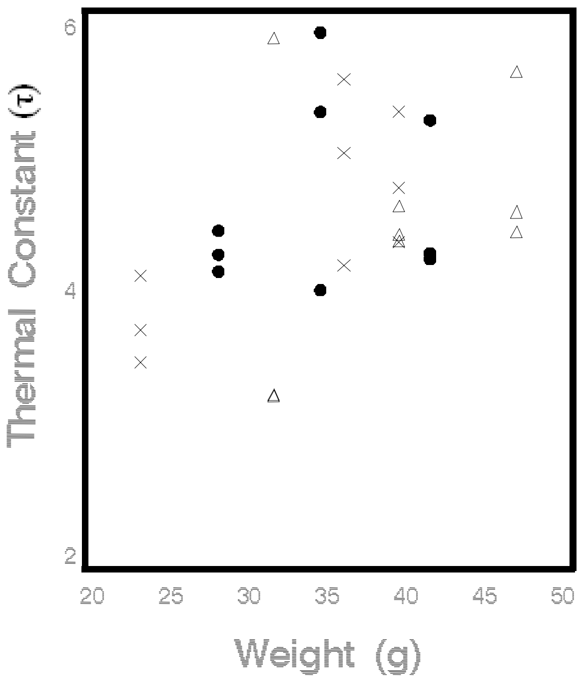

Figure 9. Thermal constants for nine male snakes (three replicates each) versus body weight (g) for light Western Terrestrial Garter Snakes, *Thamnophis elegans*, (X), dark *T. elegans* (●) and Common Garter Snakes, *T. sirtalis* (Δ). High τ values imply slower heating rates. There was no significant difference in heating rates between colour morphs or species. There was a general trend, albeit not significant, towards slower heating rates in larger animals.

<u>Behaviour</u>

The probability of *T. elegans* being visible in the enclosure was influenced by reproductive state, which in turn varied with colour morph (colour X reproductive state: $\chi^2_1 = 4.0214$, $p = 0.0449$); data were therefore divided and analysed separately by reproductive state. When snakes were gravid, there was a significantly higher probability that both light and dark *T. elegans* ($\chi^2_1 = 0.1386$, $p = 0.7097$) would be visible when T_bs were low ($\chi^2_1 = 13.8320$, $p = 0.0002$; Fig. 10a). This trend reversed, however, following birth when the same snakes, now nongravid, were more likely to be visible when T_bs were high ($\chi^2_1 = 6.4188$, $p = 0.0113$). There was also a significantly higher probability that dark nongravid *T. elegans* would be visible in the enclosure than light *T. elegans* ($\chi^2_1 = 15.4725$, $p < 0.0001$) during this time (Fig. 10b).

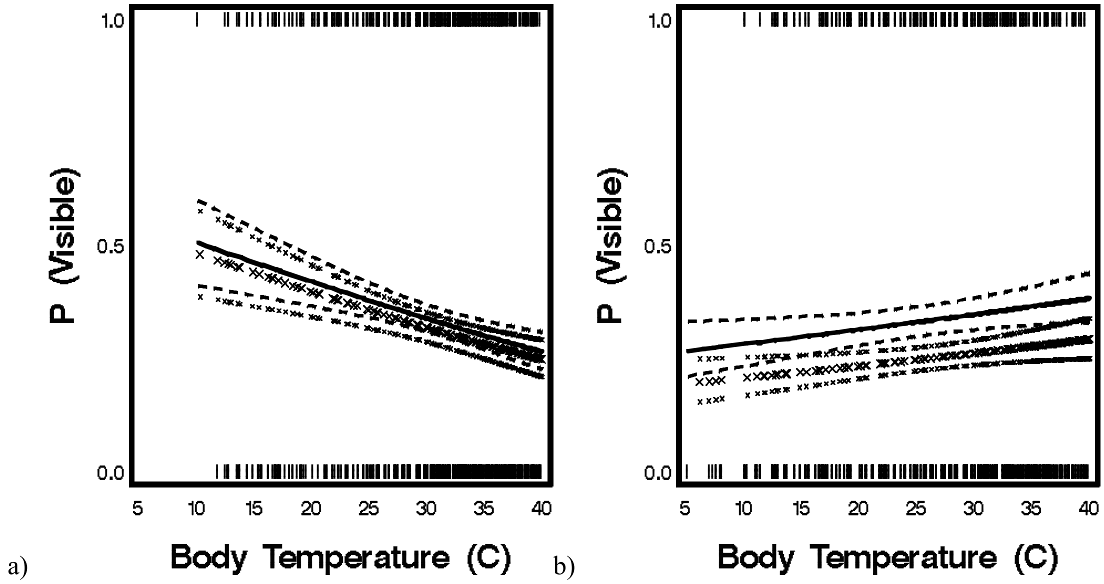

Figure 10. Probability of a) gravid and b) nongravid Western Terrestrial Garter Snakes, *Thamnophis elegans*, of both colour morphs, being visible in the outdoor enclosure vs. body temperature (T_b). The probability of being visible was significantly related, albeit in opposite directions, to T_b. Gravid snakes were more likely to be visible in the enclosure when T_bs were low and there was no significant difference between colour morphs. Nongravid snakes were more often visible when T_bs were high; dark *T. elegans* was significantly more likely to be visible. The solid black line represents the predicted values from the logistic regression and is surrounded by segmented lines representing 95% confidence limits for dark *T. elegans*. Light *T. elegans* are indicated by 'X's. The actual data points are indicated at the top and bottom of the graph.

The distance snakes maintained away from refuge in the enclosure did not vary by reproductive state ($F_{4, 30} = 1.14$, $p = 0.3355$) and so data were combined. Overall, there was no relationship between T_b and distance to cover ($F_{4, 26} = 1.76$, $p = 0.1341$). This pattern was consistent for both light and dark *T. elegans* (distance to cover X colour morph: $F_{4, 26} = 2.17$, $p = 0.0707$; Fig. 11).

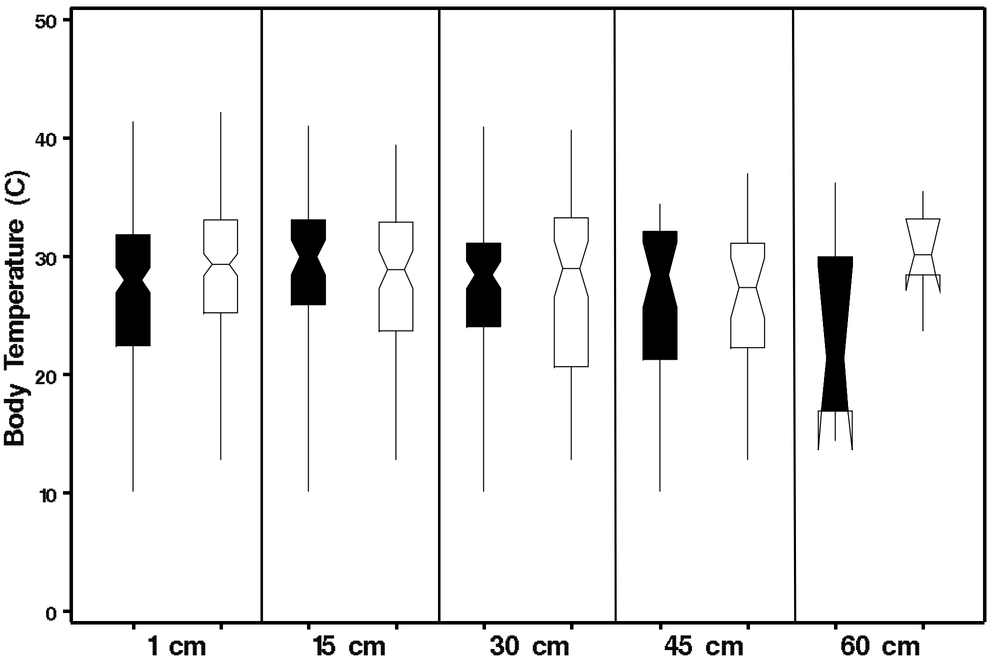

Figure 11. Summary of body temperatures (T_bs) maintained by light and dark Western Terrestrial Garter Snakes, *Thamnophis elegans*, at various distances (cm) from cover objects in the outdoor enclosure. There was no significant relationship between T_bs and distance to cover for light and dark *T. elegans* when gravid or nongravid (shown are combined values for both reproductive states). Light *T. elegans* are indicated by white boxes and dark *T. elegans* are indicated by black boxes. Each box represents 50% of the data. The median is indicated by the notches and lines extend to the minimum and maximum values.

For field captures, there was a significant interaction between T_bs and colour morph ($\chi_{1,4}$ = 5.4554, p = 0.0195) on the probability of snakes moving when first detected. There was also a similar significant interaction in the interspecific comparison (T_bs * species: $\chi_{1,4}$ = 5.4388, p = 0.0197). Data were therefore divided accordingly and separate analyses conducted for each colour morph and species. Dark *T. elegans* were more likely, albeit not significantly, to be moving when first detected at higher T_bs ($\chi_{1,2}$ = 3.2704, p = 0.0705; Fig. 12. a). Conversely, in light *T. elegans*, there was a nonsignificant negative relationship between T_bs and the probability of snakes moving when first detected in the field ($\chi_{1,2}$ = 1.7886, p = 0.1811; Fig. 12. a). Body size was included in these analyses and did not influence the probability of snakes moving when detected for either light ($\chi_{1,2}$ = 2.6008, p = 0.1068) or dark *T. elegans* ($\chi_{1,2}$ = 1.1178, p = 0.2904).

Thamnophis sirtalis was significantly more likely to be moving when first detected at higher T_bs ($\chi_{1,2}$ = 3.5555, p = 0.0593). Small *T. sirtalis* also had a higher, albeit nonsignificant, probability of moving when first seen ($\chi_{1,2}$ = 3.4130, p = 0.0647).

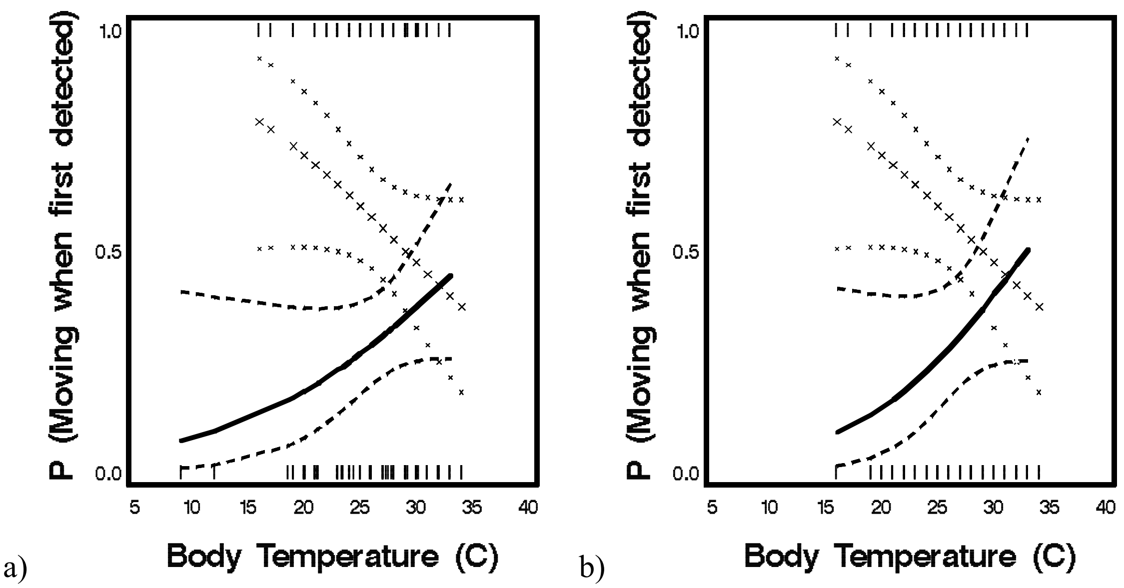

Figure 12. Probability of snakes moving when first detected in the field vs. body temperature (T_b) for a) light and dark Western Terrestrial Garter Snakes, *Thamnophis elegans*, and for b) light *T. elegans* and Common Garter Snakes, *T. sirtalis*. Warm snakes were more likely to be moving when first detected in dark *T. elegans* and *T. sirtalis*, however, the situation reversed for light *T. elegans*. The solid black lines represent the predicted values from the logistic regression and are surrounded by segmented lines representing 95% confidence limits for dark *T. elegans* in a) and *T. sirtalis* in b). Light *T. elegans* are indicated by 'X's and represent the predicted values from the logistic regression surrounded by 95% confidence limits in a) and b). The actual data points are indicated at the top and bottom of the graph.

Of the snakes that were motionless when first detected, a proportion of them did not move until capture. Unlike the previous analysis, there was no significant interaction between T_b and colour morph ($\chi_{1,4}$ = 0.1081, p = 0.7423) or T_b and species ($\chi_{1,4}$ = 0.5441, p = 0.4607) on the probability of snakes moving before capture so these interactions were deleted. The likelihood of snakes moving as they were approached was equal for dark and light *T. elegans* ($\chi_{1,3}$ = 0.1085, p = 0.7418) and for light *T. elegans* and *T. sirtalis* ($\chi_{1,3}$ = 0.5886, p = 0.4430). Colder snakes were more likely, albeit not significantly, to move before capture in both the intraspecific ($\chi_{1,3}$ = 1.3369, p = 0.2476) and interspecific ($\chi_{1,3}$ = 0.3215, p = 0.5707) comparison (Fig. 13 a & b). Smaller snakes were significantly more likely to move before capture in the intraspecific comparison ($\chi_{1,3}$ = 4.0045, p = 0.0454). Size did not, however, influence the probability of a snake moving before capture in interspecific comparison ($\chi_{1,3}$ = 2.1444, p = 0.1431).

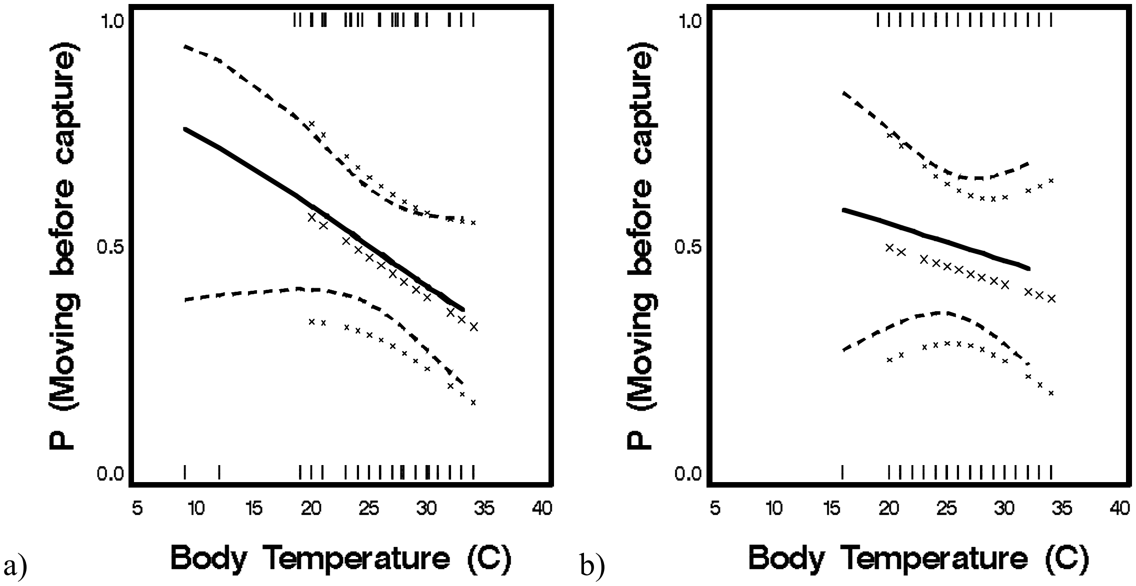

Figure 13. Probability of snakes moving before capture vs. body temperature (T_b) for a) light and dark Western Terrestrial Garter Snakes, *Thamnophis elegans,* and for b) light *T. elegans* and Common Garter Snakes, *T. sirtalis*. There was no difference in the likelihood of snakes moving before capture between either colour morphs or species. Colder snakes were more likely to move before capture, but these relationships were not significant. The solid black lines represent the predicted values from the logistic regression and are surrounded by segmented lines representing 95% confidence limits for dark *T. elegans* in a) and *T. sirtalis* in b). Light *T. elegans* are indicated by 'X's and represent the predicted values from the logistic regression surrounded by 95% confidence limits in a) and b). The actual data points are indicated at the top and bottom of the graph.

The probability of light and dark *T. elegans* evading capture was higher with increased operative temperatures (T_es; $\chi_{1,2}$ = 3.2553, p = 0.0712) and was consistent between colour morphs ($\chi_{1,2}$ = 0.2511, p = 0.6163; Fig. 14a). In the interspecific comparison, snakes in microhabitats where T_es, and presumably T_bs, were higher had a significantly higher probability of evading capture ($\chi_{1,2}$ = 5.4339, p = 0.0197). Light *T. elegans* were more likely to be captured than *T. sirtalis* ($\chi_{1,2}$ = 12.0757, p = 0.0005; Fig. 14b).

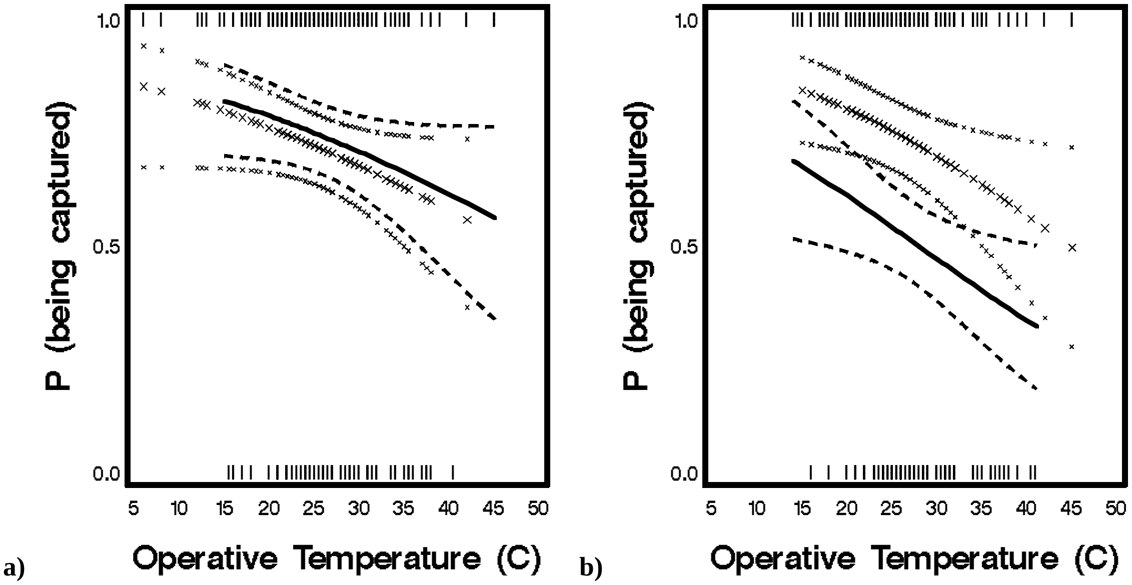

Figure 14. Probability of snakes being captured in the field vs. operative temperature (T_e) for light and dark Western Terrestrial Garter Snakes, *Thamnophis elegans*, and Common Garter Snakes, *T. sirtalis*. In general, snakes that were occupying warm areas (i.e. high T_es), and presumably had high T_bs, had a greater chance of evading capture. Light and dark *T. elegans* had an equal probability of being captured, a), whereas in the interspecific comparison, light *T. elegans* were significantly more likely to be captured, b). The solid black lines represent the predicted values from the logistic regression and are surrounded by segmented lines representing 95% confidence limits for dark *T. elegans* in a) and *T. sirtalis* in b). Light *T. elegans* are indicated by 'X's and represent the predicted values from the logistic regression surrounded by 95% confidence limits in a) and b). The actual data points are indicated at the top and bottom of the graph.

DISCUSSION

Overall, my results showed a strong relationship between body temperature (T_b) and reproductive state, as well as with some behaviour measures, but differences attributable to colour in Western Terrestrial Garter Snakes, *Thamnophis elegans,* or between light *T. elegans* and Common Garter Snakes, *T. sirtalis*, were not straightforward. Females maintained higher and less variable T_bs when gravid than nongravid in both *T. elegans* colour morphs. The operative temperature at which gravid *T. elegans* began thermoregulating (i.e. changepoint T_e) was similar between light and dark *T. elegans* and mean T_bs above this point were comparable. Dark *T. elegans*, however, had significantly higher mean T_bs below the changepoint T_e. Light *T. elegans* heated faster than dark *T.*

elegans, although the difference was not statistically significant. Gravid snakes of both colour morphs were more likely to be visible in the enclosure when T_bs were low, but nongravid dark *T. elegans* were significantly more likely to be visible when T_bs were high. In the field, dark *T. elegans* and *T. sirtalis* were more likely to be moving when first detected when T_bs were high, however, this trend was reversed in light *T. elegans*. Cold snakes, as inferred from T_es at capture/potential capture sites, of both colour morphs and species were more likely to be captured and light *T. elegans* were significantly more likely to be captured than co-occurring *T. sirtalis*.

In the enclosure experiment, gravid females of both colour morphs of *T. elegans* maintained higher and less variable T_bs than nongravid females. Although *T. sirtalis* were not included in the enclosure experiment, previous work suggests that the thermal ecology of light *T. elegans* and *T. sirtalis* in Creston are comparable (Charland 1995). Charland (1995) used similar approaches to those presented here – he monitored T_bs of gravid and nongravid light *T. elegans* and *T. sirtalis* in outdoor enclosures using radiotelemetry. Overall, he concluded that gravid females of both species maintained significantly higher mean and less variable T_bs than nongravid snakes and this trend was consistent for light *T. elegans* and *T. sirtalis*. I therefore hypothesize that gravid *T. sirtalis* would follow the same general patterns described here and exhibit thermoregulatory similarities as gravid females of light and dark *T. elegans*.

The pattern of elevated T_bs and subsequent temperature shifts following parturition has been well established in squamate reptiles (Beuchat 1986, Charland and Gregory 1990, Daut and Andrews 1993, Blouin-Demers and Weatherhead 2001, Crane and Greene 2008, Gardner-Santana and Beaupre 2009). By maintaining higher T_bs, gravid females can presumably speed embryonic development (Lourdais et al. 2004), thereby shortening gestation periods (Shine 1983, Schwarzkopf and Shine 1991). Ultimately, this can reduce the amount of time a snake experiences the various fitness costs associated with reproduction, such as reduced mobility and locomotion (Seigel et al. 1987, Olsson et al. 2000). Early birth also allows more time for the growth of neonates (Case 1978) and the accumulation of energy reserves before hibernation for females (Costanzo 1985). Maintenance of lower T_bs in nongravid females, however, is more consistent with an energy conservation strategy. Low T_bs allow snakes to reduce energetic costs, thereby allowing the investment or transfer of existing energy reserves for future reproductive benefit (Madsen and Shine 1992, Bonnet et al. 1999, Crane and Greene 2008).

The operative temperature at which gravid females began thermoregulating (changepoint $T_e = 29.15°C$, corresponding to $T_b = 32.35°C$) was similar for light and dark *T. elegans.* Although gravid snakes had access to higher T_es, 29.15°C was the point at which the slope of T_b vs. T_e changed, suggesting the regulation of T_bs above this T_e in both colour morphs. Above the changepoint T_e, light and dark gravid *T. elegans* maintained similar high mean T_bs which appears to be a shared strategy that does not differ with colour. The most likely explanation for these observations is that, when T_es were high and gravid females had access to a wide variety of temperature regimes, females selected T_bs that provided the best possible embryonic developmental temperatures (Charland and Gregory 1990, Peterson et al. 1993) that could result in optimal offspring phenotypes (Shine and Downes 1999, Wapstra 2000). Several studies have shown that offspring traits are optimized at specific incubation temperatures. For example, gravid females of the viviparous skink, *Eulamprus tympanum,* maintained a specific developmental temperature (T_b = 32°C), despite the higher temperatures made available in the laboratory (Schwarzkopf and Shine 1991). Laboratory studies of California populations of *T. elegans* revealed that embryonic survival was maximized at T_b = 26.6°C, which corresponded to the lowest levels of developmental abnormalities and matched T_bs maintained by free-ranging snakes (O'Donnell and Arnold 2005). Although this specific value (i.e. T_b = 26.6°C) does not correspond to the changepoint T_b measured here (i.e. T_b = 32°C), presumably this value could represent a similar type of critical temperature for optimal development of snakes in BC.

Dark *T. elegans* maintained significantly higher mean T_bs in the enclosure when ambient temperatures were low. Higher mean T_bs have been observed in the melanistic forms of the Common Adder, *Vipera berus* (Forsman 1995) and more generally in the dark morphs of invertebrate groups such as bees (Pereboom and Biesmeijer 2003), beetles (DeJong et al. 1996), butterflies (Van Dyck and Matthysen 1998), and grasshoppers (Forsman 2001) where colour variation occurs. In *T. sirtalis*, melanistic individuals maintained higher mean T_bs than striped conspecifics and this thermal advantage was realized during the colder parts of the active season (Gibson and Falls 1979). In mammals, higher T_bs associated with dark colours during cold periods has also been demonstrated in springboks, *Antidorcas marsupialis*, that vary in pelt colouration (Hetem et al. 2009). What are the potential benefits associated with higher T_bs during cold periods, particularly for snakes? Perhaps dark snakes have an improved ability to convert solar radiation to body heat, resulting in higher T_bs and associated faster speeds. As a result, these snakes may be better able to acquire resources (i.e. increased foraging success because of increased speed) when ambient temperatures are low, such as early in the season when maintaining elevated T_bs is presumably most challenging. This advantage may be particularly important for snakes that live in high-latitude environments where activity periods and thermoregulatory opportunities can be limited (Isaac and Gregory 2004).

Light *T. elegans* heated up faster, albeit not significantly, than dark *T. elegans*. This result was contrary to expectation and to numerous studies documenting heating differences in colour dimorphic populations of snakes (Gibson and Falls 1979, Forsman 1995, Tanaka 2005), beetles (Brakefield and Wilmer 1985), bees (Pereboom and Biesmeijer 2003), grasshoppers (Forsman et al. 2002), and butterflies (Watt 1968, Van Dyck and Matthysen 1998) where dark individuals generally heat faster. The ability to heat quickly is advantageous because thermoregulation can be energetically expensive given that individuals have to shuttle between microhabitats for basking and also potentially risky if basking exposes individuals to predators. So, individuals that are able to take advantage of short basking opportunities that can facilitate speed of digestion, development of young, or increased locomotory abilities would potentially experience a fitness advantage.

It is unclear why heating rates did not vary between light *T. elegans* and *T. sirtalis*. This result was unexpected given that greater heat flow, and hence, higher temperatures, has been demonstrated in the dark skin of *T. sirtalis* (Gibson and Falls 1979). The colour variable lizard, *Lacerta dugesii*, does not, however, exhibit different heating rates associated with colour (Crisp et al. 1979) and this lack of relationship has also been observed in some snakes (Bittner et al. 2002) and beetles (DeJong et al. 1996).

So, perhaps the lack of difference between light *T. elegans* and *T. sirtalis* males is real or it might reflect limitations in experimental design – this experiment was restricted to a small number of male snakes and perhaps subtle differences could not be detected statistically. Future experiments of this type should be repeated to include gravid and nongravid females to characterize potential differences that may be attributable to sex or reproductive state.

Heating rates were also generally higher for small-bodied snakes and this is a trend that has been demonstrated in various taxa (Heinrich 1986, Pereboom and Biesmeijer 2003, Tanaka 2005, Rice et al. 2006). Body size has the ability to influence the speed at which individuals raise their T_bs and as result, should influence the behaviours used to regulate T_bs (Rice et al. 2006) as well as other ecological functions (Forsman et al. 2002). Ultimately, rapid heating rates will have profound influence on small-bodied snakes, which may also experience other selective pressures such as increased predation (Webb and Whiting 2005).

Interestingly, both dark *T. elegans* and *T. sirtalis* were more likely, albeit not significantly, to be moving when first detected in the field when T_bs were high. Light *T. elegans*, on the other hand, had a higher probability of moving when seen when T_bs were low. Cold snakes are typically slower (Stevenson et al. 1985), but elsewhere I show that light *T. elegans* were generally faster at lower T_bs whereas both dark *T. elegans* and *T. sirtalis* were generally faster at higher T_bs (see Chapter 4). Although light *T. elegans* may be faster and more inclined to be moving when detected at lower T_bs, snakes were still more likely to be captured than *T. sirtalis* across a wide range of T_es, suggesting that other antipredator behaviours may be important (see Chapter 4). These findings indicate that the relationship between colour and antipredator behaviours is complex and can be mediated by T_b and may be of particular ecological and evolutionary importance, especially among ectothermic animals (Forsman and Appelqvist 1998).

Surprisingly, there were no relationships between T_bs, colour, and the distances snakes maintained relative to cover in the enclosure. I hypothesized that being away from cover would be risky because the chances of being captured during a long retreat would be high. As a result, I expected that snakes should reduce these risks as much as possible and if they had to be away from cover, they would do so when their T_bs were high and they would be fast. Although this relationship was not realized in my work, such trends have been exhibited in the Keeled earless lizard, *Holbrookia propinqua*, where lizards were found farther away from cover as substrate temperatures increased (Cooper 2000). In iguanids, for example, lizards decreased their distance from shrubs early in the day when T_bs were low and increased their distance from cover later in the day as temperatures rose (Bulova 1994).

The subjective assessment of potential ‘cover’ available for snakes in the enclosure may have introduced inaccuracy into distance estimates thereby making detection of trends difficult. I would expect, however, that this concern would be minimized in the enclosure where cover objects were obvious and few in numbers. On the other hand, there may be no relationship between T_bs and distance to cover and snakes may rely on other behaviours to prevent detection or evade predators. Alternatively, snake behaviour may be affected less by distance to cover and more related to previous exposure to predators and subsequent assessments of risk. For example, the behaviour of *Craugastor* sp. frogs was shown to vary with the amount of previous experience with terrestrial and avian predators (Cooper et al. 2008). Although it is difficult to gauge actual predator exposure in natural systems, a novel approach would be to incorporate this factor (i.e. by using 'naïve' snakes) and evaluate the resulting effects on antipredator behaviours.

The extensive set of behavioural observations of snakes in the outdoor enclosures suggests that snakes behaved 'naturally' in these semi-natural environments. Snakes exhibited behaviours similar to those of free-ranging snakes, such as retreating when disturbed and basking near cover objects, and I therefore assume that these behaviours and T_bs that were also measured during this time were similar to those of free-ranging snakes under the same conditions. Although the direct extrapolation of experimental results to field estimates may not always be straightforward (Irschick et al. 2005), thermal studies of Northern Water Snakes, *Nerodia sipedon*, demonstrated that mean T_bs were similar when measured in captivity and in the wild (Brown and Weatherhead 2000) suggesting that extrapolating from enclosure to field studies is a valid approach.

It is unclear why the changepoint T_e for nongravid females could not be precisely determined in the enclosure experiment. One likely explanation is that T_bs were more variable following birth and as a result, the detection of general trends was made difficult. Previous work using the same statistical approach concluded that nongravid female European grass snakes, *Natrix natrix*, exhibited a lower, but not significantly different, changepoint T_e than gravid females (Isaac and Gregory 2004). We might expect a comparable result for the nongravid snakes studied here but further work should emphasize the temperature monitoring of more nongravid snakes in order to increase sample sizes and improve the discriminatory ability of statistical tests.

Although the mechanisms or behaviours used by snakes to maintain T_bs was not the focus of study, there were no apparent differences in thermoregulatory behaviour, at least in terms of visibility in the outdoor enclosure. Gravid females of both colour morphs exhibited similar patterns of visibility, presumably in attempts to maximize basking opportunities. When snakes became nongravid, however, dark *T. elegans* were more likely to be visible in the enclosure, particularly when T_bs were high. These results suggest that the likelihood of snakes being visible and at risk to visually-oriented predators may be influenced by snake colour, which presumably varies with reproductive condition. Colour-specific behaviours have been observed in nongravid *V. berus* in which melanistic snakes were significantly more likely to be visible than striped conspecifics (Forsman 1995). As a result, these dark-coloured snakes maintained higher T_bs. In free-ranging Japanese four-lined snakes, *Elaphe quadrivirgata*, the thermoregulatory strategy (i.e. habitat use) changed between colour morphs when ambient temperatures became cool (Tanaka 2007). In order to develop a comprehensive profile of thermoregulatory behaviour and the potential tradeoffs that may occur with colour in garter snakes, future research should quantify the frequency and duration of basking periods as well as the amount of activity or frequency of movements employed by snakes to pursue basking opportunities.

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CHAPTER 3

ARE GARTER SNAKES CONSPICUOUS TO THEIR PREDATORS? COMPARING CHROMATIC AND ACHROMATIC CRYPSIS IN GARTER SNAKES FROM THE PERSPECTIVE OF MAMMALS AND BIRDS.

INTRODUCTION

Colour influences numerous aspects of the ecology of organisms, including foraging ability (Hanlon et al. 1999), thermoregulation (Bittner et al. 2002), communication with conspecifics (Endler 1987) and defense against predators (Losey et al. 1997). To avoid being preyed upon, animals can use bright, contrasting colours to advertise their unpalatability or cryptic, concealing colours to minimize their detection (Edmunds 1974). The use of cryptic colours to avoid detection is widespread in diverse taxonomic groups found in both terrestrial and aquatic environments (Kettlewell 1956, Merilaita 1998, Hanlon et al. 1999, Wente and Phillips 2003, Stuart-Fox et al. 2006).

Crypsis is considered an adaptation against visually-oriented predators whereby animals become indistinguishable from the surrounding background. Numerous mechanisms have been proposed to reduce an animal’s detectability including disruptive colouration (Stevens et al. 2006), transparency (Johnsen 2001), masquerade (Ruxton et al. 2004), mirroring (Johnsen and Sosik 2003), and countershading (Braude et al. 2001). Because of the many organisms that resemble their environment either by selecting matching backgrounds (Wente and Phillips 2003, 2005) or by changing colours to match their surrounding environment (Packard and Sanders 1971, Kats and van Draft 1986, Hanlon and Messenger 1988, Kelman et al. 2006), background matching is often regarded as the principal method of attaining crypsis. Background matching refers to the visual similarity between an animal and its environment and it requires that both the colour and pattern of the animal prevent detection. Currently, there is no broadly applied approach to quantify and analyze the role of pattern elements in crypsis. As such, this study focuses on background matching from a colour matching perspective; that is, from the two perceptual channels that animals use in parallel, namely colour, or chromatic cues, and brightness, or achromatic cues (Jacobs 1985, Vorobyev and Osorio 1998).

Crypsis via background matching is closely linked to the resemblance of an animal to its surrounding habitat and it can be expected that the better an animal matches its background, the less its chance of being detected by a predator. Background matching can become difficult, however, for animals that have fixed colouration and use different habitats. Snakes, for example, typically use a variety of microhabitats in which to forage, find mates, bask, and take refuge (Slip and Shine 1988, Charland and Gregory 1994, Blouin-Demers and Weatherhead 2001). If background matching is closely linked to the resemblance of an animal to its surrounding habitat and multiple habitat types are frequently used, is optimal colouration (colour that confers maximum advantage), still possible? Merlaita (1999) used mathematical modeling approaches in a simplified heterogeneous environment to demonstrate that crypsis can be optimized either by adopting a compromised colouration to two habitats or conversely, by adopting colouration to one background type. The tradeoff between these two strategies depends, however, on a number of factors such as the behaviour of prey and predators (Houston et al. 2007) and the relative frequency of different habitat types (Merilaita et al. 1999, Houston et al. 2007). Recent computer-based experiments investigating the evolution of crypsis concluded, however, that compromised colouration results in overall poor survivorship, indicating that strategies for optimal concealment are multifaceted (Sherratt et al. 2007).

Early studies of animal colouration involved quantifying colours that were visible to human observers (Poulton 1890, Cott 1940) by ranking colours into arbitrary classifications (Sweet 1985, Agawa and Kawata 1995, Nicoletto 1995, Forsman 1999) and by matching colours to commercial standards (Endler 1984, Braude et al. 2001, Weiss 2002). Animal crypsis has also been quantified not by evaluating colour *per se* but by using the time taken for human observers to detect individuals (Cuadrado et al. 2001, Cooper et al. 2008). Relying on human assessments of colour and crypsis is problematic for numerous reasons. First, humans are not visually sensitive to shorter wavelengths (UV: 300-400nm) and the use of subjective approaches may completely underestimate the significance of these wavelengths (Bennett et al. 1994, Majerus et al. 2000). Second, the subjective techniques employed assume that colour can be scored in an accurate and repeatable fashion whereas measurement error, in this case arising from human bias, has been shown to influence the classification of other subjective measures (Eden et al. 2007) and may be important in this context. And third, human vision may lack the sensitivity or acuteness necessary to detect biologically important colour differences (Aviles 2008, Ryer et al. 2008). Conversely, human assessments may attach significance to colour differences when in fact these differences may not be functionally significant (Martin et al. 2009). Despite these fundamental limitations, human-biased approaches are still utilized in animal signaling research (Fitzstephens and Getty 2000, Hoffman and Blouin 2000, Cooper et al. 2008, Ryer et al. 2008, Manriquez et al. 2009, Todd et al. 2010). It is therefore critical to evaluate and interpret achromatic and chromatic data from the perspective of relevant potential predators (Wilson et al. 2007, Aviles 2008, Defrize et al. 2010).

In this study, which focuses on snakes, the main predators are birds and mammals. The mechanism by which chromatic and achromatic signals are interpreted by these predators depends on the eye's sensitivity to certain wavelengths of light. For example, birds are tetrachromatic, indicating that they have four types of visual cells, or photoreceptors, in the eye that are used to discriminate light wavelengths (Hart 2001). Avian vision can then be further divided into two visual types based on the very short-wavelength sensitivity of photoreceptors: birds having ultraviolet-sensitive (UVS) photoreceptors (e.g. passerines and gulls) and those having violet-sensitive (VS) photoreceptors (Oden and Hastad 2003). Birds with a VS visual pigment can perceive light in the UV range, but they are less sensitive to this wavelength of light than UV-sensitive birds. Mammals, on the other hand, lack sensitivity to either UV or V light and their visual sensitivity varies from monochromatic (Abrams 1987, Jacobs and Deegan) to dichromatic (Calderone and Jacobs 2003) to trichromatic (Wyszecki and Stiles 1982).

In this paper, I investigate chromatic and achromatic crypsis both within and between species of garter snakes in British Columbia, Canada. I evaluate crypsis in different populations of the Western Terrestrial Garter Snake, *Thamnophis elegans*, that exhibit geographic variation in colouration. Extensive life history studies have been completed for dimorphic *T. elegans* where two colour morphs (i.e. referred to as lakeside and meadow ecotypes) occur sympatrically (Bronikowski and Arnold 1999, Bronikowski 2000). Although the colours of the two Californian ecotypes may superficially resemble the light and dark morphs of this study, it is unknown how crypsis compares.

I also compare crypsis between two species of garter snake that vary in colour, but occur in the same geographic region – the light-coloured morph of *T. elegans* and the Common Garter Snake, *Thamnophis sirtalis*. I interpret, or model, the chromatic and achromatic data of garter snakes and their backgrounds from the perspective of a mammalian predator (ferret) and two potential avian predators (i.e. V-type and UV-type). I also model the data from the perspective of a human observer to evaluate the possibility of differential snake crypsis to the human visual system. In this chapter I ask the following three questions:

1. Do snakes select basking sites and occur in habitats that maximize chromatic and achromatic crypsis?

Do snakes bask on backgrounds and occur in habitats that increase their concealment from potential predators? I predicted that crypsis would be maximized in backgrounds where snakes were found basking, suggesting selection for microhabitats where detection is minimized. I also predicted that crypsis would be greatest in habitats where snakes normally occur vs. the habitats of the other colour morphs or species.

2. Can predators differentially detect colour morphs and species based on achromatic and chromatic crypsis?

Because we know that visual ability differs between animals, are snake colour morphs and species differentially cryptic to mammal and bird predators? I predicted that crypsis would vary between the different visual models. More specifically, I predicted that snakes would be more cryptic to bird predators because of their enhanced visual sensitivity and acuity.

3. Are snakes differentially cryptic to human observers?

In my experience, it is more difficult to locate light *T. elegans* in the field than either dark *T. elegans* or *T. sirtalis*, presumably because the latter are less cryptic. I therefore predicted that crypsis to the human visual system would vary between colour morphs and species of snakes.

METHODS

Study area, snake capture & measurements

I completed field work in the spring and summer of 2008 at three study sites in British Columbia (BC), Canada where the light and dark morph of the Western Terrestrial Garter Snake, *Thamnophis elegans,* and the Common Garter Snake, *T. sirtalis*, occur (see Chapter 1, Fig. 1). I searched for snakes at the three sites on foot and captured snakes by hand. Several variables were measured on each snake I caught (see Chapter 2 & 4); those relevant to this study included body length (snout-vent length, SVL) and sex. In addition, each individual's reproductive condition was assessed by gently palpating adult females in the abdomen to determine whether they were gravid. I individually marked each snake for future identification by clipping a unique combination of subcaudal scutes on the underside of the tail to ensure each snake was included only once in this crypsis study.

Sampling for crypsis

Snakes were used to evaluate crypsis if they met the following predetermined criteria: 1. motionless and coiled in a fixed position when detected, indicative of typical basking behaviour, and 2. fully visible from overhead (Fig. 1). I used these two criteria as indicators that the snakes had actively selected the sites where they were found for basking, rather than being caught there incidentally as they were just passing by in search of food, retreat sites, etc. Snakes that were visible from overhead were at risk from potential avian predator attempts from above and could obviously be seen by mammal predators on the ground. If snakes met these criteria, they were taken to the laboratory where the colour (or spectral reflectance) and brightness (or luminance) of their dorsal surface were measured. Thus, this study focuses on the role of crypsis in selection of basking sites by snakes, not its role in general habitat selection.

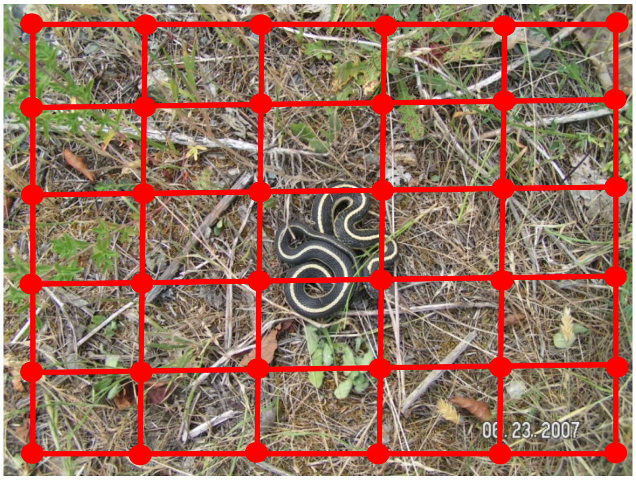

Figure 1. Photograph of a dark Western Terrestrial Garter Snake, *Thamnophis elegans*, dark morph, illustrating the two criteria required for snakes to be included in this crypsis study. Each snake must be: 1. motionless and coiled, and 2. fully visible when viewed from overhead. Also shown here is the imaginary 50cm X 50cm grid centred around each captured snake to delineate habitat samples.

Habitat samples

Because crypsis is a relative measure that depends upon the background against which animals are seen, I collected samples of the habitat surrounding each snake. For each capture, I collected 36 habitat samples from a 50cm X 50cm grid positioned symmetrically around where the motionless snake was found and divided into 10-cm intervals both horizontally and vertically (Fig. 1). I gathered habitat samples that included herbaceous vegetation, twigs, sand, rocks, etc. at every 10-cm intersection point within the grid, putting each of these 36 samples into separate paper envelopes. Where there were multiple samples of similar habitat types, such as a large area of grass, I collected only one sample and copied the data the appropriate number of times.

Following this, I collected habitat samples from a similar grid that was located in a random plot near the capture site. These plots were positioned at a 2-m distance from the point of capture in one of the four cardinal directions, which was randomly chosen. All 72 habitat samples for a given snake were taken back to the laboratory, where I measured the spectral reflectance of each item.

Spectral reflectance of snakes and their surrounding habitat

Prior to any reflectance measurements being collected, I pre-determined the number of scans required to capture a comprehensive profile of dorsal colour variation for each colour morph of *T. elegans* and *T. sirtalis*. This initial step was required because reflectance measurements are collected according to the relative proportion of different dorsal colours. To do this, I photographed the dorsal surface of a random sample of 9 snakes (3 each of light *T. elegans*, dark *T. elegans,* and *T. sirtalis*) in the laboratory, using a Nikon D200 digital SLR camera (Nikon Inc.; Fig. 2).

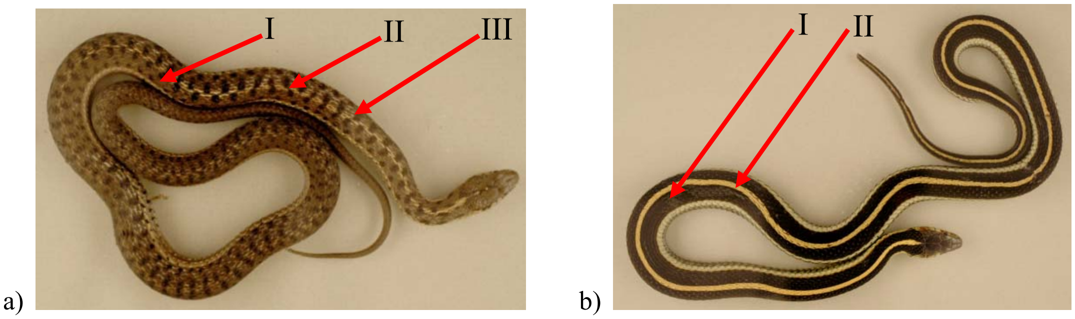

Figure 2. Dorsal photographs taken of a) light Western Terrestrial Garter Snake, *Thamnophis elegans*, and b) Common Garter Snake, *T. sirtalis,* in the laboratory. These photographs were processed with ImagePro 6.0 to quantify the area of the various dorsal colour elements. Not shown here is dark *T. elegans*, which closely resembles the dorsal photograph of *T. sirtalis.* I = dorsal colour, II = dorsal stripe, III = dorsal patch.

Next, I used the digital software package, ImagePro Plus 6.0 (Media Cybernetics Inc., 2006), to analyse each digital image and calculate the area of each colour region

(Table 1). I quantified (by outlining) the dorsal surface colour, stripe and blotching and divided these values by the total dorsal area to determine the percentage area of each colour type. This procedure was repeated for the three individuals of each colour morph and species. Following this, I calculated mean values for each colour morph and species (e.g. mean percentage area of dorsal colour, stripe, blotch for the light morph, etc.), which were then used to determine the number of spectral scans required for each region (Table 1). Given the amount of time required to collect one reflectance scan, I predetermined that 20 scans would be taken of each individual. Thus, if the dorsal colour comprised 85% of the dorsal surface area, then 17 of the 20 scans would be taken of the dorsal colour along the length of the snake. The same logic would apply to the stripes and blotches for all colour morphs and species.

Table 1. Summary of the number of scans required for each section of dorsal colour as determined by image analysis for light and dark Western Terrestrial Garter Snakes, *Thamnophis elegans*, and Common Garter Snakes, *T. sirtalis*. Twenty scans were chosen to create a body colouration profile and were partitioned based on the percent dorsal area of each colour type.

Colour morph/species	**Dorsal Component**	**amount of total dorsal area (%)**	**# of scans for each colour morph/species (of 20 total)**
Light *T. elegans*	Dorsal colour	85%	17
	Dorsal stripe	10%	2
	Dorsal blotch	5%	1
Dark *T. elegans*	Dorsal colour	85%	17
	Dorsal stripe	15%	3
T. sirtalis	Dorsal colour	85%	17
	Dorsal stripe	15%	3

I then took a second round of photographs on a smaller subset of individuals (1 each of light & dark *T. elegans* and *T. sirtalis*) using a Nikon D200 digital SLR camera (Nikon, Inc.) fitted with a UV-pass filter. This arrangement allowed me to determine whether snakes were reflective in the UV-range of the electromagnetic spectrum. That is, any bright regions on the images would represent areas that are reflective in the UV because only UV light can pass through the filter. None of the snakes were reflective in the UV range (Fig. 3), so I did not require the use of an illumination source that produced light in the UV and could instead use equipment that generated light only from the visible to near-infrared region of the electromagnetic spectrum.

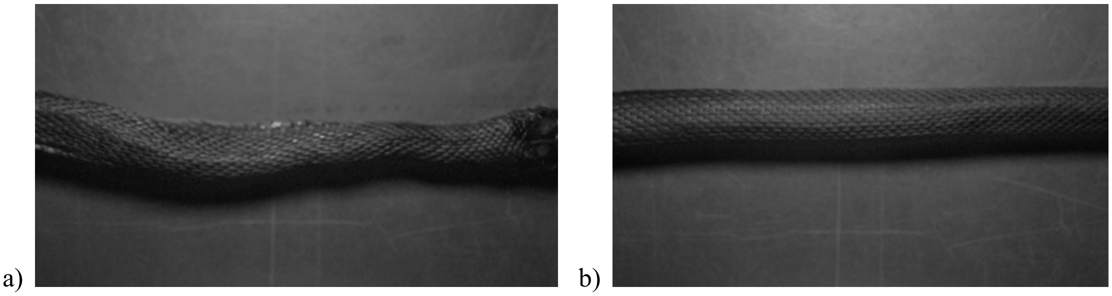

Figure 3. Dorsal photographs of a) light Western Terrestrial Garter Snake, *Thamnophis elegans*, and b) Common Garter Snake, *T. sirtalis* taken in the laboratory. Snakes were illuminated with a black light (UV light only) and images were captured with a Canon D200 digital camera affixed with a UV-*pass* filter. Colours will reflect light (i.e. show up as white) if they are reflective in the ultraviolet range of the light spectrum. These photographs confirm that the various dorsal colour elements were not reflective in the UV. Not shown here is the dorsal photograph of dark *T. elegans*, which closely resembles the *T. sirtalis* image.

Following the above-described process, I measured the spectral reflectance of all snakes and habitat samples using a reflectance probe (R200-7-UV/VIS, Ocean Optics, Dunedin, FL, USA) connected to a dual-channel spectrophotometer (SD2000, Ocean Optics) and a halogen light source (LS-1, 360nm to 2000nm, Ocean Optics). All measurements were recorded using a notebook computer (Dell Inspiron 9400) operating the Ocean Optics' SpectraSuite software (Ocean Optics). Prior to each reflectance scanning session, the spectrophotometer was calibrated against a mercury argon light source (HG-1, Ocean Optics) using a 100μm fibre (QP100-2-UV/VIS; Ocean Optics).

All spectra were obtained in a dimly lit room to reduce the amount of ambient light that might influence measurements. The reflectance probe was maintained at a 90 degree angle to the dorsal surface and was kept at a constant 2 mm from all snakes and habitat samples (Fig. 4). Immediately prior to each reflectance measurement, I scanned a matte white standard (WS-1, Ocean Optics) and removed the dark current from the signal to take into account any potential noise introduced by these measurements. I collected reflectance scans for one snake at a time by gently restraining it under the reflectance probe; if at any point the animal appeared to be in distress (e.g. excessive movements, striking, etc.), measurements were stopped and the animal was allowed to rest for at least one hour. Soon after measurements were completed, I released snakes at their point of capture under favourable weather conditions.

Figure 4. Photograph illustrating how spectral measurements were collected in the laboratory. Spectrophotometer and light source are shown in the background (far right) with the reflectance probe leading from those units to the sample (i.e. snake or habitat sample) situated on the platform. The position (angle and distance) of the reflectance probe was maintained constant by holding the probe in place with clamps on the stand. All data are collected using the SpectraSuite program (Ocean Optics) shown on the computer.

Irradiance

I measured downwelling irradiance (μmol m^{-2} s^{-1} nm^{-1}) using a direct-attach cosine-correcting probe (CC-3-DA, Ocean Optics, Dunedin, FL, USA) and a spectrophotometer (SD2000, Ocean Optics). Prior to the field season, the irradiance sensor was calibrated with an optical radiation calibrator (LI-1800-02; Li-Cor, Lincoln, NE) by Dr. Craig Hawryshyn (Queen's University). Irradiance measurements were collected shortly after each capture in the field to assure the same light conditions as at the time of capture (Fig.

5). Three irradiance scans were taken for each capture and the resulting mean spectrum was used to transform reflectance values (i.e. 20 per snake and 72 habitat samples per capture) to radiance values (μmol m^{-2} s^{-1} $nm^{-1}sr^{-1}$) by multiplying values by mean irradiance.

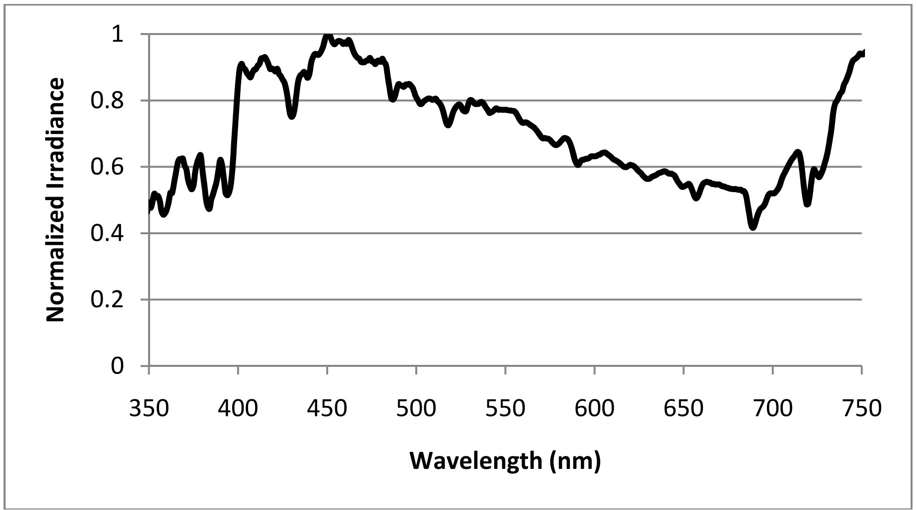

Figure 5. Spectra of normalized irradiance, or ambient light (μmol m^{-2} s^{-1} nm^{-1}), measured on August 10, 2008 following a snake capture. This spectrum is normalized to a peak of 1 for display purposes only. Mean irradiance values were used to convert the reflectance values of snake and habitat samples to their respective radiance values, which were used in all visual modeling.

Visual Modeling

In total, I constructed four visual models from which to interpret garter snake colouration. The ferret (*Mustelo putorious furo*) model was constructed using microspectrophotometry (MSP) data (i.e. λ_{max} values for each photoreceptor type) obtained from Calderone & Jacobs (2003) and the human model was constructed using MSP data obtained from Wyszecki & Stiles (1982). I then converted these data to Gaussian visual pigment absorbance curves following approaches outlined in Govardovskii *et al*. (2000; Fig. 6 a & b). Ferret and human photoreceptors do not possess oil droplets, therefore, no modification of the resulting absorbance spectra was required.

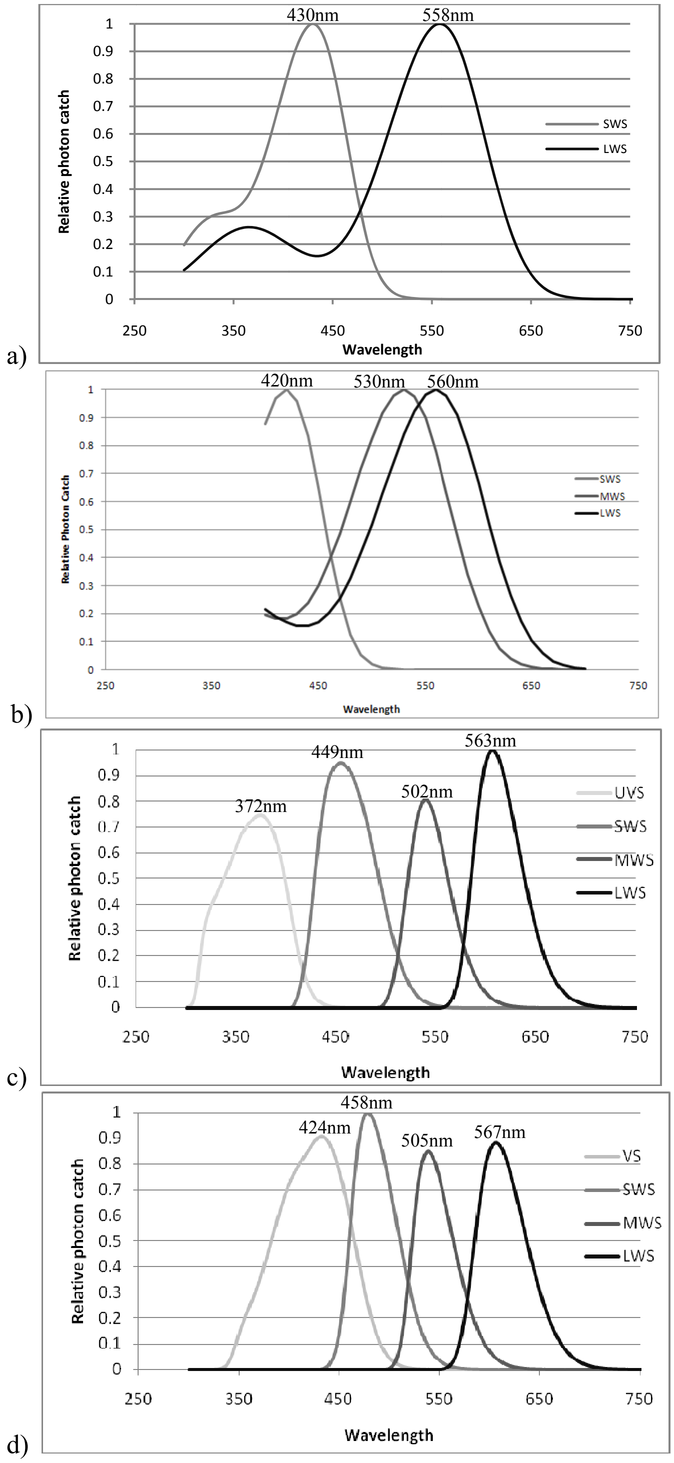

Figure 6. Photon catch values for cone classes of a) ferret (*Mustelo putorious furo*), b) human (*Homo sapiens*), c) UV-type bird (Blue tit, *Parus major*), and d) V-type bird (Indian peafowl, *Pavo cristatus*). Values indicate the maximum absorption wavelengths (λ_{max}) of each cone class (wavelength sensitivity of ultraviolet=UVS, violet=VS, short =SWS, medium=MWS, and long=LWS photoreceptors).

Two bird visual models were constructed using data provided by Nathan Hart (Fig. 6 c & d). Although MSP data have not been collected for all bird species (Hart 2001, Hart and Vorobyev 2005), opsin gene sequencing work by Oden and Hastad (2003) suggests that potential snake predators such as large-bodied passerines and gulls have visual systems that are more sensitive to ultraviolet light (or UVS), whereas other predatory birds such as hawks, falcons, and corvids are more sensitive to violet light (or VS). Visual modeling for these two visual types is often represented by two ‘model’ species for which detailed MSP data have been collected, namely the blue tit, *Parus caeruleus*, a UVS bird, and the Indian peafowl, *Pavo cristatus*, a VS bird. Bird photoreceptors possess oil droplets, therefore, all photoreceptor data for the two bird models were corrected for the modifying effects of these oil droplets as well as for the effects of other ocular media (e.g. cornea; as per Hart, *pers. comm.*).

I then estimated the response of the four visual models by multiplying the spectral sensitivity of each cone class (two cone classes for the dichromatic ferret, three cones for the trichromatic human, and four cones for the tetrachromatic birds) by each radiance spectrum (20 of each snake and 72 associated habitat samples). To do this, I derived the photoreceptor quantum catches (Q_i) of each cone class following the formula described in Endler & Mielke (2005):

$$Q_i = \int_{\lambda\mathrm{min}}^{\lambda\mathrm{max}} R(\lambda)I(\lambda)SS\,(\lambda)d(\lambda)$$

where λ represents wavelength, R(λ) is the reflectance spectrum of the item being viewed, I(λ) is the irradiance spectrum or ambient light illuminating the item, and SS (λ) is the spectral sensitivity of a given photoreceptor type, all integrated over a wavelength of interest, d(λ).

The working assumption, as outlined by Fleishman and Persons (2001), is that neural stimulation from each of the cones is equal in response to a white stimulus. To meet this assumption, the resulting values were summed for each cone class and each sum was multiplied by the reciprocal of the area under the spectral sensitivity curves of each associated photoreceptor to account for the differences in area under the sensitivity curves.

Colour perception

To compare an entire set of colour values, we can treat the photoreceptor output of a given patch (a single scan in this case) as a multivariate value, or vector. Therefore, multivariate statistics can be used to compare a ‘cloud’ of colour scans or vectors. The perception of colour patches by the three different predator visual models is defined by the relative stimulation values $\{S\}$, where $\{S\}=\{S_{sws}$ and $S_{lws}\}$ for the ferret model, $\{S\}=\{S_{uvs}, S_{sws}, S_{mws}, S_{lws}\}$ for the UV-type bird model and $\{S\}=\{ S_{vs}, S_{sws}, S_{mws}, S_{lws}\}$ for the V-type bird model (Endler and Mielke 2005). Following Endler & Mielke (2005), an object with N patches (i.e. a snake with 20 colour scans is N=20) comprises a N X 2 matrix, **S**, for the ferret model or a N X 4 matrix for the two bird models, where $\{S\}$ represents the rows of the matrix. This **S** matrix is then converted to a compositional matrix, $\mathbf{S_c}$, by dividing each of the cone outputs in **S** by its row total (i.e. $u= S_{UVS}/ S_{UVS} + S_{SWS}+S_{MWS}+S_{LWS}$, or, $v= S_{VS}/ S_{VS} +S_{SWS}+S_{MWS}+S_{LWS}$; $s= S_{SWS}/S_{UVS}$ or $S_{VS}+S_{SWS}+S_{MWS}+S_{LWS}$; $m= S_{MWS}/S_{UVS}$ or $S_{VS}+S_{SWS}+S_{MWS}+S_{LWS}$; $l= S_{LWS}/S_{UVS}$ or $S_{VS}+S_{SWS}+S_{MWS}+S_{LWS}$).

For the two bird models, the next step is to convert each $\mathbf{S_c}$, or set of u/v, s, m, and l values as described above, to tetrahedral coordinates $\{\mathbf{S_t}\} = \{x, y, z\}$ using the following formulas as described in Endler & Mielke (2005):

$$x = \frac{1-2s-m-u}{2}\sqrt{\frac{3}{2}} \quad y = \frac{-1+3m+u}{2\sqrt{2}} \quad z = u - \frac{1}{4}$$

where u (or v)= photoreceptor output of the ultraviolet-(or violet-) wavelength-sensitive cone, s = photoreceptor output of the short-wavelength-sensitive cone, m= photoreceptor output of the medium-wavelength-sensitive cone, and l= photoreceptor output of the long-wavelength-sensitive cone. The result is that every stimulus is represented as a point that lies within tetrahedral colour space.

Because ferrets are dichromatic and humans are trichromatic, conversion of points to tetrahedral colour space is inappropriate. I converted ferret cone outputs to single data points using the formula: $x = S_{sws} / S_{sws} + S_{lws}$. The result is that every stimulus value, rather than falling within tetrahedral colour space, is represented as a point that falls on a 2-dimensional plane (Endler, *pers. comm.*). I converted human cone outputs to two data points using the formula: $x = S_{mws} + S_{lws} / 2$; $y = (\sqrt{3}/2) \text{ X } S_{lws}$ resulting in points that fall within a 3-dimensional space (Endler, *pers. comm.*).

This approach results in a description of snake colouration as $\{S_t = x, y, z\}$ for the two bird models, $\{S_t = x\}$ for the ferret model, and $\{S_t = x, y\}$ for the human visual model. Similarly, a second group of colour points each with comparably sized colour parameters (i.e. *x, y, z* for birds, *x* for ferrets, and *x, y* for humans) describes the surrounding habitat. The two sets of colour coordinates can then be compared using compositional-type analyses. Distances between the clouds of colour points in the tetrahedral space as well as along the 2- and 3-dimensional plane can therefore be interpreted as perceived differences among the colours.

Brightness perception

The mechanisms that control achromatic signals in animals are not fully understood, however, evidence suggests that double cones, containing the LWS pigment, are probably involved in brightness discrimination in birds (Osorio et al. 1999, Hart 2001). Double cones contain two structures: the principal and accessory members, each of which contain the LWS pigment, but differ in their oil droplet characteristics (Hart 2002). I calculated the mean absorbance curve for the two oil droplet types and multiplied this by the absorbance spectra of the LWS pigment.

In animals that lack double cones, such as mammals, it is presumed that LWS cones function in achromatic discrimination (Osorio and Vorobyev 2005). I therefore used the λ_{max} value for the LWS pigment ($\lambda_{max=}$558nm) obtained from Calderone & Jacobs (2003) and Wyszecki & Stiles (1982). I then modeled the data following approaches outlined in Govardovskii *et al*. (2000).

Comparing entire colour patterns

The distribution of colour coordinates is such that it violates numerous multivariate statistical assumptions such as normality and homogeneity of variances. Because of this, I used a distribution-free statistical approach, LSED-MRPP, that measures differences between colour patches in any aspect of their distributions, such as differences in mean, variance, and skewness (Endler and Mielke 2005). LSED-MRPP yields an effect size measure called ‘disparity’ or ‘K’ and increased divergence between groups that differ in their distributions results in higher ‘K’ values. This approach has been broadly applied in work on colour conspicuousness in various taxonomic groups, including birds (Endler et al. 2005), fish (Kemp et al. 2008), lizards (Macedonia et al. 2009), and pythons (Wilson et al. 2007).

The analytical approach used here compares differences in animal and background colours from the perspective of various viewing organisms by calculating colour differences in multivariate space. Unlike other visual modeling methods, such as Vorobyev et al.'s (1998) model (referred to as the V-O model), this compositional approach assumes that all cone classes contribute equally to colour perception. In addition, another simplifying assumption is that photoreceptor noise, or the variability induced by the mechanisms of light transduction, is not taken into account (Vorobyev and Osorio 1998). Also, the compositional analysis applied here could be described as a more conservative approach because it may not have the precision to differentiate between spectrally similar colours, particularly at low light levels. This limitation is minimized, however, in garter snakes, in which diurnal activity (hence high light levels) is more common.

Despite these limitations, the main advantage of the disparity method is that it compares an entire set of colour points, or quantum catches, rather than being restricted to the discrimination of pairs of spectral points. Because the background colour surrounding garter snakes in this study is typically heterogeneous, one would have to arbitrarily pick two values to compare if the V-O model were employed. I therefore argue that the compositional approach is more appropriately used here because it includes all colour elements in proportion to how they are represented both on the snake and in the surrounding habitat.

I calculated disparity values for each snake against the respective background on which it was found basking as well as for the same snake against the background of a nearby random habitat sample. I then repeated this approach for all light and dark *T. elegans* and *T. sirtalis* from the perspective of the four visual models. In this and all subsequent statistical analyses, chromatic and achromatic values were calculated separately and 'K' values were treated as the dependent variable. I evaluated whether snakes selected basking sites that maximized achromatic crypsis by comparing disparity values using paired t-tests, in which the independent variable was background type (i.e. snake against selected basking background vs. same snake against corresponding random background). I then performed comparable analyses investigating chromatic crypsis, but used the non-parametric Wilcoxon signed-rank test (SAS 1988) due to the heterogeneous distribution of the data. I conducted separate tests for light and dark *T. elegans*, and *T. sirtalis* for each visual model (UV-type bird, V-type bird, ferret, and human).

Next, I evaluated whether crypsis was maximized on the backgrounds where snakes were normally found by comparing 'K' values of snakes in their typical basking habitats to 'K' values of the same snakes compared against the basking habitats of the other colour morph or species. In both the achromatic and chromatic analyses, snakes were randomly paired with associated backgrounds of the other colour morph or species. I used paired t-tests for the achromatic analyses and Wilcoxon signed rank tests for the chromatic analyses because of the heterogeneous distribution of the data. In both cases, habitat type was treated as the independent variable and I conducted separate analyses for each combination of colour morph and visual model.

I then evaluated differential crypsis between colour morphs and between species for both brightness and colour. Because achromatic crypsis (and chromatic crypsis) were calculated for the same snake using different visual models, the data were not statistically independent. I therefore used univariate 2-way analysis of variance (ANOVA) models with individuals nested within colour morph/species to compare mean crypsis (dependent variable) between individuals, colour morphs/species, and visual models (independent variables). Colour morph/species and visual model were treated as fixed-effects factors and individuals nested within colour morph/species were treated as a random-effects factor.

Finally, using independent samples t-tests, I tested whether a human observer could differentially detect the colour morphs/species based on achromatic crypsis. I then repeated the analysis, using the Mann Whitney U test, to evaluate if humans could differentially detect colour morphs/species based on chromatic crypsis.

All statistical tests and graphing were performed using SAS 9.2. Post-hoc model comparisons were performed using the Tukey-Kramer method and I assessed significance at $\alpha = 0.05$. All animal maintenance and experimental procedures complied with guidelines for live reptiles and were approved by the University of Victoria Animal Care Committee.

RESULTS

In total, I captured 39 snakes that fit the criteria for crypsis analysis – 12 dark Western Terrestrial Garter Snakes, *Thamnophis elegans*, 13 light *T. elegans*, and 13 Common Garter Snakes, *T. sirtalis*. Samples included adult males, nongravid and gravid females ranging from 395mm to 645mm (Table 2). Analyses could not be divided by sex or reproductive condition because of small sub-samples.

Table 2. Summary of mean body size (SVL, mm; ± SD) and number of snakes used in the crypsis study divided by sex and reproductive condition for light and dark Western Terrestrial Garter Snakes, *Thamnophis elegans*, and Common Garter Snakes, *T. sirtalis*.

Colour morph/species	**Males**	**Nongravid Females**	**Gravid Females**
Dark *T. elegans*	441.67 ± 21.01 mm (n=3)	544.00 ± 45.68 mm (n=4)	591.00 ± 53.73 mm (n=5)
Light *T. elegans*	434.50 ± 21.92 mm (n=2)	482.00 ± 36.77 mm (n=3)	488.63 ± 16.72 mm (n=8)
T. sirtalis	478.50 ± 0.71 mm (n=2)	505.60 ± 78.40 mm (n=5)	568.83 ± 21.12 mm (n=6)

Question #1: Do snakes select sites and occur in areas that maximize crypsis?

In general, achromatic crypsis was similar between basking sites that were selected and nearby random backgrounds for light and dark *T. elegans* as well as for *T. sirtalis* (Fig. 7 a to c). These similarities were consistent across all three predator visual models (Table 3).

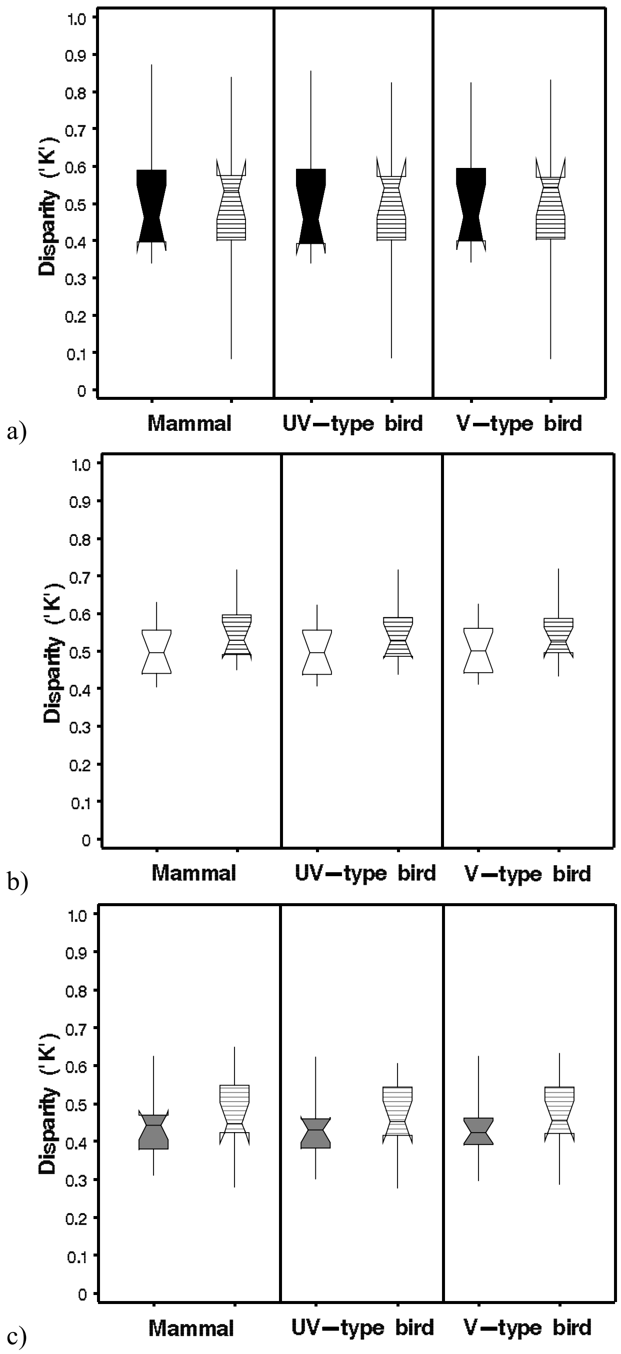

Figure 7. Achromatic crypsis (Disparity values, 'K') in backgrounds where snakes selected for basking (solid colour) vs. nearby random backgrounds (hatched lines) for a) dark Western Terrestrial Garter Snakes, *Thamnophis elegans*, b) light *T. elegans*, and c) Common Garter Snakes, *T. sirtalis*. Shown are 'K' values from the perspective of three predator models: mammal (ferret, *Mustelo putorious furo*), UV-type bird (Blue tit, *Parus major*), and V-type bird (Indian peafowl, *Pavo cristatus*). Overall, there was no difference in achromatic crypsis between snakes in capture sites vs. same snakes in nearby random sites from the perspective of the three predator models. Each box represents 50% of the data, the median is indicated by notches, and lines extend to the minimum and maximum values.

Table 3. Summary of paired t-tests and Wilcoxon signed rank tests comparing achromatic and chromatic crypsis (Disparity values, 'K') in selected basking sites vs. random habitats for dark and light Western Terrestrial Garter Snakes, *Thamnophis elegans*, and Common Garter Snakes, *T. sirtalis*. Comparisons were made from the perspective of the three predator visual models- mammal, UV-type bird and V-type bird model. There was no difference in achromatic crypsis between the three predator models, whereas, dark and light *T. elegans* were significantly more chromatically cryptic to the UV-type and V-type bird model.

Crypsis Type	**Species/Colour morph**	**Mammal (Ferret, *Mustelo putorious furo*)**	**UV-type bird (Blue tit, *Parus major*)**	**V-type bird (Indian peafowl, *Pavo cristatus*)**
Achromatic	Dark *T. elegans*	t_{11} = -0.07, p = 0.5267	t_{11} = -0.18, p = 0.5715	t_{11} = -0.21, p = 0.5799
Achromatic	Light *T. elegans*	t_{12} = -1.80, p = 0.9512	t_{12} = -1.68, p = 0.9411	t_{12} = -1.63, p = 0.9360
Achromatic	*T. sirtalis*	t_{12} = -1.06, p = 0.8448	t_{12} = -1.12, p = 0.8567	t_{12} = -1.09, p = 0.8522
Chromatic	Dark *T. elegans*	S = -23, p = 0.0771	**S = -32, p = 0.0093**	**S = -34, p = 0.0049**
Chromatic	Light *T. elegans*	S = -8.5, p = 0.5879	**S = -34.5, p = 0.0134**	**S = -33.5, p = 0.0171**
Chromatic	*T. sirtalis*	S = -6.5, p = 0.6848	S = -26.5, p = 0.0681	S = -23.5, p = 0.1099

In contrast to achromatic crypsis, chromatic crypsis was generally greater (i.e. lower disparity values, 'K') in selected backgrounds where snakes were basking than in corresponding random backgrounds. This trend held for all colour morphs and visual models, but the strength of these differences varied between visual models (Table 3). That is, both dark and light *T. elegans* were significantly more chromatically cryptic in selected backgrounds from the perspective of the two bird models (Fig. 8 a & b). *Thamnophis sirtalis* also was more chromatically cryptic against selected backgrounds, but not significantly so (albeit close in the case of the UV-type bird, Table 3; Fig. 8 c).

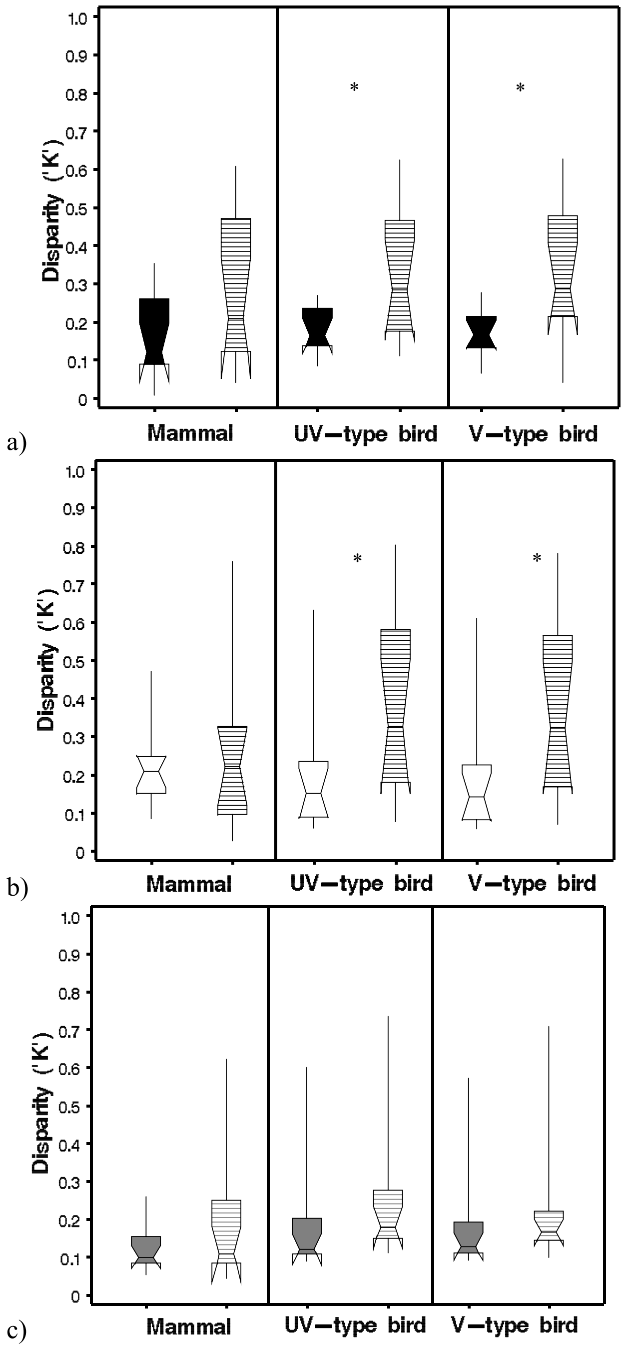

Figure 8. Chromatic crypsis (Disparity values, 'K') in backgrounds where snakes selected for basking (solid colour) vs. nearby random backgrounds (hatched lines) for a) dark Western Terrestrial Garter Snakes, *Thamnophis elegans*, b) light *T. elegans*, and c) Common Garter Snakes, *T. sirtalis*. Shown are 'K' values from the perspective of three predator models: mammal (ferret, *Mustelo putorious furo*), UV-type bird (Blue tit, *Parus major*), and V-type bird (Indian peafowl, *Pavo cristatus*). Dark and light *T. elegans* were significantly more cryptic in terms of colour to the UV-type and V-type bird model. Significant differences between pairs are indicated by '*'. Each box represents 50% of the data, the median is indicated by notches, and lines extend to the minimum and maximum values.

Although not statistically significant, dark *T. elegans* and *T. sirtalis* were more achromatically cryptic in habitats where they normally occurred than they would have been if compared against the habitats of the light *T. elegans* (Table 4; Fig. 9 a and d). Contrary to expectation, light *T. elegans* were more achromatically cryptic in habitats of the dark *T. elegans* as well as in the habitats of *T. sirtalis* (Table 4; Fig. 9 b and c) than in the habitats where they themselves occurred.

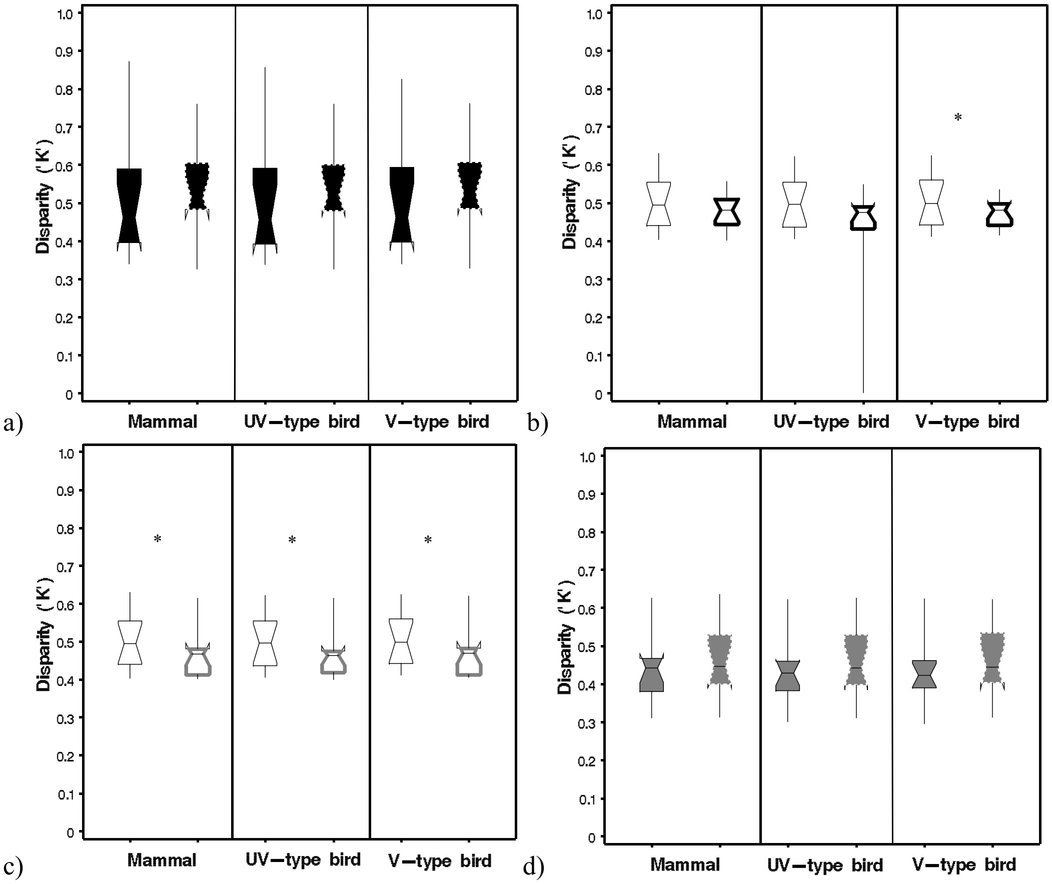

Figure 9. Achromatic crypsis (Disparity 'K' values) for a) dark Western Terrestrial Garter Snakes, *Thamnophis elegans*, when *in situ* (solid black) vs. habitat of light *T. elegans* (black surrounded by white dashed line), b) light *T. elegans* when *in situ* (solid white) vs. habitat of dark *T. elegans* (white surrounded by solid black line), c) light *T. elegans* when *in situ* (solid white) vs. habitat of Common Garter Snakes, *T. sirtalis* (white surrounded by solid grey line), and d) *T. sirtalis* when *in situ* (solid grey) vs. habitat of light *T. elegans* (grey surrounded by solid white dashed line). Shown are 'K' values from the perspective of three predator models: mammal (ferret, *Mustelo putorious furo*), UV-type bird (Blue tit, *Parus major*), and V-type bird (Indian peafowl, *Pavo cristatus*). Overall, achromatic crypsis of dark *T. elegans* and *T. sirtalis* was greater in habitats where they occurred, but this trend did not hold for light *T. elegans.* Significant differences between pairs are indicated by '*'. Each box represents 50% of the data, the median is indicated by notches, and lines extend to the minimum and maximum values.

Table 4. Summary of paired t-tests and Wilcoxon signed rank tests comparing achromatic and chromatic crypsis (Disparity values, ‘K’) in habitats where dark and light Western Terrestrial Garter Snakes, *Thamnophis elegans*, and Common Garter Snakes, *T. sirtalis,* were normally found vs. habitats of different colour morphs or species. Comparisons were made from the perspective of the three predator visual models- mammal, UV-type bird, and V-type bird model. Overall, achromatic crypsis of dark *T. elegans* and *T. sirtalis* was greater in habitats where they occurred. Light *T. elegans*, on the other hand, were more achromatically cryptic when compared to the habitats of dark *T. elegans* and T. *sirtalis.* Snakes of both colour morphs and species were more chromatically cryptic, albeit not significantly, in habitats where they normally occurred than in habitats of the opposing colour morph or co-occurring species.

Crypsis Type	**Comparison**	**Mammal (Ferret, *Mustelo putorious furo*)**	**UV-type bird (Blue tit, *Parus major*)**	**V-type bird (Indian peafowl, *Pavo cristatus*)**
Achromatic	Dark *T. elegans* when *in situ* vs. habitat of light *T. elegans*	t_{11} = -1. 09, p = 0.8497	t_{11} = -1.28, p = 0.8862	t_{11} = -1.74, p = 0.9449
Achromatic	Light *T. elegans* when *in situ* vs. habitat of dark *T. elegans*	t_{12} = 1. 42, p = 0.0910	t_{12} = 1.56, p = 0.0762	**t_{12} = 1.78, p = 0.0502**
Achromatic	Light *T. elegans* when *in situ* vs. habitat of *T. sirtalis*	**t_{12} = 1. 86, p = 0.0439**	**t_{12} = 1.96, p = 0.0367**	**t_{12} = 1.98, p = 0.0355**
Achromatic	*T. sirtalis* when *in situ* vs. habitat of light *T. elegans*	t_{12} = -1. 70, p = 0.9430	t_{12} = -1.85, p = 0.9554	t_{12} = -1.77, p = 0.9487
Chromatic	Dark *T. elegans* when *in situ* vs. habitat of light *T. elegans*	S = -14, p = 0.3013	S = -17, p = 0.2036	S = -19, p = 0.1514
Chromatic	Light *T. elegans* when *in situ* vs. habitat of dark *T. elegans*	S = -7.5, p = 0.6355	S = -14.5, p = 0.3396	S = -13.5, p = 0.3757
Chromatic	Light *T. elegans* when *in situ* vs. habitat of *T. sirtalis*	S = -12.5, p = 0.4143	S = -10.5, p = 0.4973	S = -9.5, p = 0.5417
Chromatic	*T. sirtalis* when *in situ* vs. habitat of light *T. elegans*	S = -8.5, p = 0.5879	S = -12.5, p = 0.4143	S = -16.5, p = 0.2734

Snakes of both colour morphs and species were more chromatically cryptic in habitats where they normally occurred than in habitats of the opposing colour morph or co-occurring species (Fig. 10 a to d). Although these differences are not statistically significant, the general trend suggests that dark and light *T. elegans* and *T. sirtalis* were more chromatically cryptic in their respective habitats (Table 4).

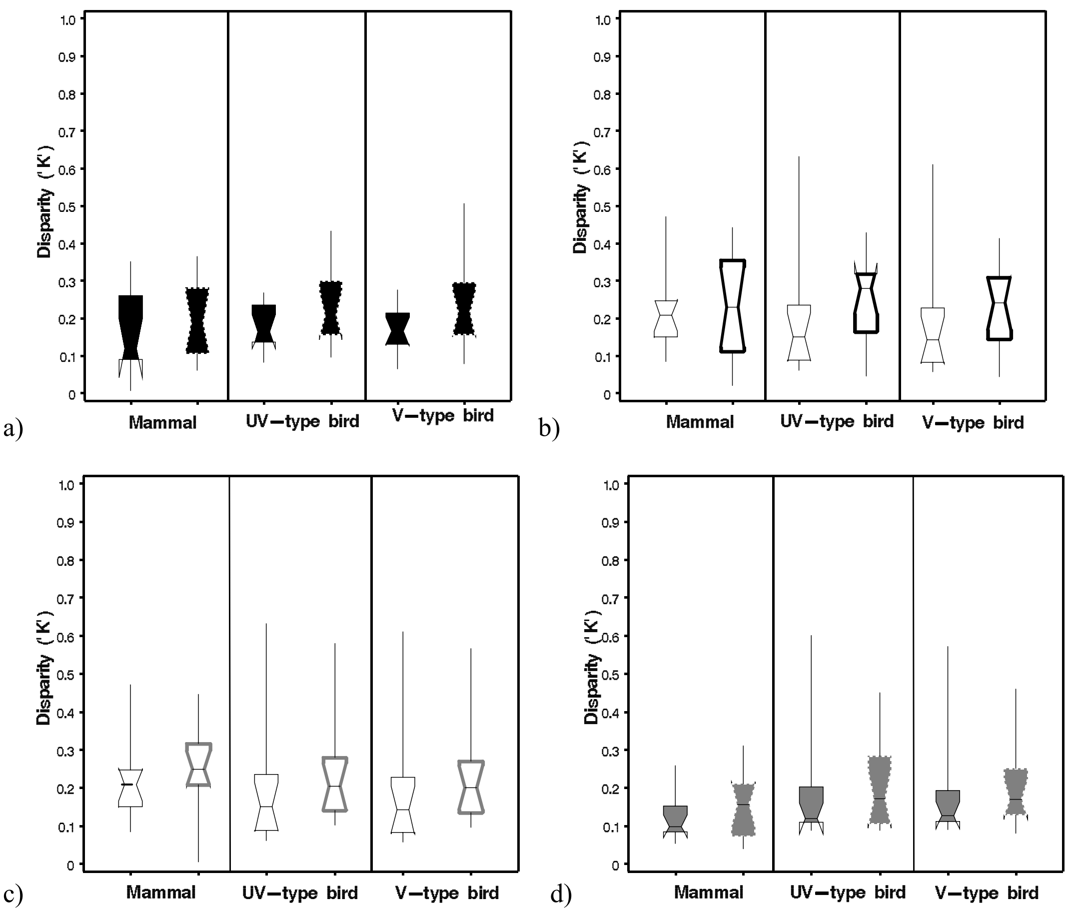

Figure 10. Chromatic crypsis (Disparity values, 'K') where a) dark Western Terrestrial Garter Snakes, *Thamnophis elegans,* occurs *in situ* (solid black) vs. habitat of light *T. elegans* (black surrounded by white dashed line), b) light *T. elegans* occurs *in situ* (solid white) vs. habitat of dark *T. elegans* (white surrounded by solid black line), c) light *T. elegans* occurs *in situ* (solid white) vs. habitat of Common Garter Snakes, *T. sirtalis* (white surrounded by solid grey line), and d) *T. sirtalis* occurs *in situ* (solid grey) vs. habitat of light *T. elegans* (grey surrounded by solid white dashed line). Shown are 'K' values from the perspective of three predator models: mammal (ferret, *Mustelo putorious furo*), UV-type bird (Blue tit, *Parus major*), and V-type bird (Indian peafowl, *Pavo cristatus*). Snakes of both colour morphs and species were more chromatically cryptic in habitats where they normally occurred than in habitats of the opposing colour morph or co-occurring species. Each box represents 50% of the data, the median is indicated by notches, and lines extend to the minimum and maximum values.

Question #2: Do snakes exhibit differential crypsis to mammal and avian predators?

In the intraspecific comparison, the interaction between visual model and colour morph was nonsignificant for both achromatic ($p = 0.2145$) and chromatic ($p = 0.6397$) crypsis and was therefore deleted from the analysis. The similar interaction between visual model and species in the interspecific comparison was also nonsignificant for achromatic ($p = 0.3301$) and chromatic ($p = 0.3623$) crypsis and was also removed. As expected, there was significant variability between individuals in achromatic ($p < 0.0001$) and chromatic ($p = 0.0018$) crypsis as seen by mammals, UV-type birds, and V-type birds in the intra-specific comparison. Similarly, the same level of variability occurred between individuals in achromatic ($p < 0.0001$) and chromatic ($p = 0.0022$) crypsis as seen by the three predator models in the interspecific comparison.

Dark and light *T. elegans* were equally cryptic ($F_{1,48} = 0.69$, $p = 0.4118$) from an achromatic perspective to the bird and mammal predators ($F_{2,48} = 0.67$, $p = 0.5180$; Fig. 11 a). Both colour morphs also exhibited similarity in chromatic crypsis ($F_{1,48} = 1.71$, $p = 0.1967$) to the three predators ($F_{2,48} = 0.05$, $p = 0.9471$; Fig. 11b). Light *T. elegans*, on the other hand, was significantly less achromatically cryptic than *T. sirtalis* ($F_{1,50} = 2599.44$, $p < 0.0001$; Fig. 11 a). Achromatic crypsis also varied by visual model ($F_{2,50} = 4.16$, $p = 0.0213$). Post hoc analyses showed that snakes exhibited similar crypsis to peacocks (V-type birds) and ferrets and between bluetits (UV-type birds) and ferrets – snakes were significantly more achromatically cryptic to UV- than V-type birds. In terms of colour, light *T. elegans* was less cryptic, albeit not significantly, than *T. sirtalis* ($F_{1,50} = 2.96$, $p = 0.0917$) and there was no difference in chromatic crypsis between the predator models ($F_{2,50} = 0.12$, $p = 0.8901$; Fig. 11 b).

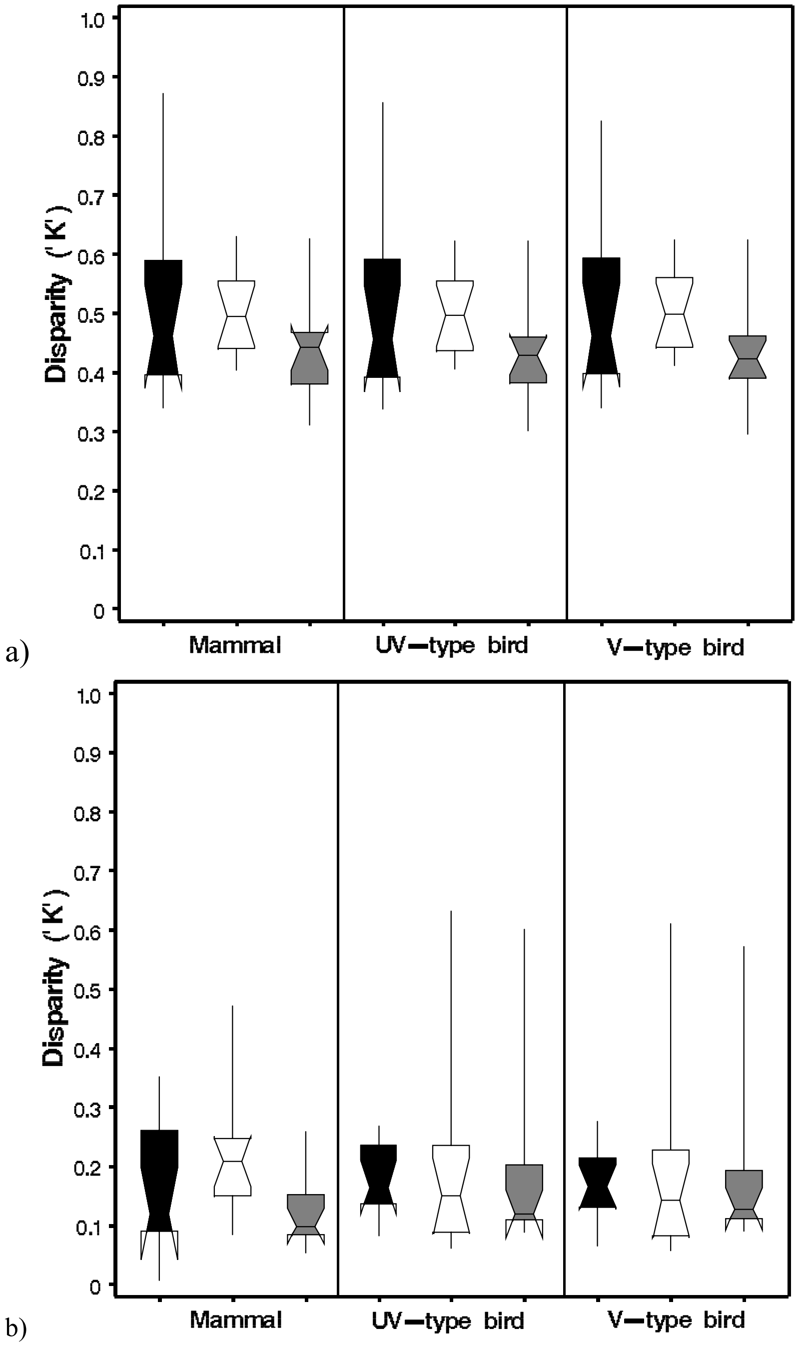

Figure 11. Comparison of a) achromatic and b) chromatic crypsis (Disparity values, 'K') between dark Western Terrestrial Garter Snakes, *Thamnophis elegans*, light *T. elegans*, and Common Garter Snakes, *T. sirtalis* as seen by the mammal, UV-type bird, and V-type bird model. Overall, snakes were more achromatically cryptic to the UV-type bird. Dark and light *T. elegans* were equally achromatically and chromatically cryptic to the three predator models. Light *T. elegans* was less achromatically and chromatically cryptic than *T. sirtalis* to all visual models, however, the strength of difference varied. Dark *T. elegans* = black boxes, light *T. elegans* = white boxes, *T. sirtalis* = grey boxes. Each box represents 50% of the data, the median is indicated by notches, and lines extend to the minimum and maximum values.

Question #3: Are snakes differentially cryptic to human observers?

Achromatic crypsis did not differ between the two colour morphs of *T. elegans* from the perspective of the human observer ($t_{14.5} = -0.22$, $p = 0.8284$). In terms of colour, light *T. elegans* was significantly less cryptic than dark *T. elegans* to the human model ($Z = -2.094$, $p = 0.0362$). In the interspecific comparison, light *T. elegans* was significantly less achromatically ($t_{24} = -2.45$, $p = 0.0217$) and chromatically cryptic ($Z = 2.462$, $p = 0.0138$) to humans than the co-occurring *T. sirtalis*.

DISCUSSION

The application of objective methods to collect, analyse and interpret crypsis using the visual systems of potential predators is perhaps one of the most important contributions of the research described here. This approach is in contrast to most other research on snakes in which subjective methods have been used to quantify and evaluate the importance of colour pattern variation (Jackson et al. 1976, Sweet 1985, Brodie 1989, King 1992, Shine et al. 2003, Niskanen and Mappes 2005, Webb and Whiting 2005, Brown et al. 2007). The research presented in this chapter begins to address at least some of the concerns with using subjective methods, by using the objective quantification of the colour of snakes and their backgrounds using spectrophotometry and the assessment of snake crypsis from the perspective of mammal and avian predators using visual modeling approaches. The results suggest that dark and light Western Terrestrial Garter Snakes, *Thamnophis elegans*, and Common Garter Snakes, *T. sirtalis,* were found basking on backgrounds that maximized chromatic crypsis, although the strength of these differences varied with predator type. Both colour morphs of *T. elegans* and *T. sirtalis* were more cryptic in terms of colour and brightness in their respective habitats, whereas this was not the case for achromatic crypsis for light *T. elegans*. Dark and light *T. elegans* appeared equally cryptic in terms of colour and brightness to both bird and mammal models, whereas light *T. elegans* was less achromatically and chromatically cryptic than *T. sirtalis* to all predator models. There was also evidence in the interspecific comparison to suggest that snakes were differentially cryptic to predator types and less achromatically cryptic overall to UV-type bird predators. And finally, light *T. elegans* was significantly less achromatically cryptic than *T. sirtalis* and less chromatically cryptic to both dark *T. elegans* and *T. sirtalis* from the perspective of human observers.

Crypsis is a function of the contrast of an animal to its surrounding background (Endler 1978). On backgrounds where snakes were found basking vs. nearby randomly chosen backgrounds, snakes were more chromatically cryptic to all three predator models. This trend held for both colour morphs and species, suggesting that snakes select sites for basking that maximize crypsis, at least in terms of colour. Early research demonstrated the close relationship between animal and background colours in a variety of amphibians and reptiles (Norris and Lowe 1964). Since this extensive correlational work, empirical research has demonstrated active selection for matching backgrounds in other taxa such as spiders (Heiling et al. 2005), moths & butterflies (Moss et al. 2006, Shreeve 2008), cephalopods (Chiao and Hanlon 2001), and a variety of anurans (Tordoff 1980, Morey 1990, Heinen 1993, Wente and Phillips 2003, 2005). Background matching also has been shown in snakes – juvenile green tree pythons, *Morelia viridis,* which can be either red or yellow in colour, selected habitats at the ground level near the rainforest edge where they were most cryptic and, as green adults, moved to interior rainforest canopy habitats, where crypsis was maximized (Wilson et al. 2007). However, this strong correlation between the colour of animals and their backgrounds does not appear to be universal (Brattstrom and Warren 1955, Gray 1978, Capula and Luiselli 1995, Shine et al. 2003, Wente and Phillips 2005). In addition to crypsis, other conflicting factors might influence background matching, such as sexual differences in microhabitat use (Merilaita and Jormalainen 1997) degree of predation risk experienced in different microhabitat types (Houston et al. 2007), and thermoregulatory constraints (Adolph 1990, Ahensjo and Forsman 2006). Nonetheless, there is growing evidence to support the hypothesis that active selection of matching backgrounds operates in at least some animal groups and the broad applicability of these results across different snake taxa remains to be tested.

What then is the adaptive significance, if any, for microhabitat selection that maximizes crypsis? Although crypsis is an intuitively accessible concept (Bond 2007), demonstrating its function and quantifying the effects on fitness has proven difficult. Researchers often hypothesize that crypsis is an effective anti-predator strategy (Cott 1940, Belk and Smith 1996, Hanlon et al. 1999, Wauters et al. 2004, Hoekstra et al. 2005), but in order to demonstrate the adaptive function of cryptic colouration, studies must illustrate how crypsis contributes to fitness. Research, mostly in insects, has demonstrated how crypsis can contribute to increased survivorship (Cott 1940, Kettlewell 1956, Edmunds 1974, Gillis 1982, Sandoval 1994, Losey et al. 1997, Forsman and Appelqvist 1999, Lyytinen et al. 2004, Ahensjo and Forsman 2006). In vertebrates, however, similar types of research showing a selective advantage for colour matching are lacking. Empirical demonstrations in live animals and plasticine replicas suggest that conspicuous forms of some fish (Endler 1980), birds (Gotmark and Hohlfalt 1995), mammals (Vignieri et al. 2010), amphibians (Tordoff 1980, Morey 1990), and snakes

(Andren and Nilson 1981, Bittner 2003, Niskanen and Mappes 2005) can be non-randomly preyed upon. In some cases, the relationship between colour and predation can depend on differences between ages/sizes (King 1993) or between sexes (Forsman and Shine 1995). Differential selection pressure can therefore play a role in the maintenance of colour variation both within (Tordoff 1980, Morey 1990, Vignieri et al. 2010) and between populations (Hoekstra and Nachman 2003, Nachman et al. 2003). Ultimately, colour-biased predation can have important implications for the evolution of animal colouration, at least in those species in which a genetic mechanism controlling colour occurs (Ritland et al. 2001, Hoekstra and Nachman 2003, King 2003, Hoekstra et al. 2004, Mundy et al. 2004, Rosenblum et al. 2004).

A reasonable working hypothesis is that *T. elegans* and *T. sirtalis* actively selected basking sites on the basis of crypsis in response to strong selection pressure in the form of risk of predation. Although the research presented here pertains to selection for cryptic basking sites, this assumes that all other factors in nature are equal and this is not necessarily the case. We might expect costs to occur if optimal crypsis occurs at a time or in a place where alternative activities cannot be pursued and certain habitats cannot be used (i.e. opportunity costs) or if the interaction with the environment is costly (Ruxton et al. 2004). As a result, we should expect tradeoffs to occur between crypsis and other behaviours, such as communication with conspecifics (Endler 1987) or foraging behaviour. For example, air temperature and crypsis interact to influence foraging-site selection in short-toed treecreepers, *Certhia brachydactyla* (Carrascal et al. 2001). Under warm conditions, treecreepers foraged in shaded areas where crypsis was high, but foraged in sunny areas when conditions were cool and crypsis was poor, suggesting that these birds trade off between being visible to predators and behaviourally thermoregulating under certain air temperature regimes. In snakes, a classic example of tradeoffs between crypsis and thermal ecology occurs in the colour polymorphic Common Adder, *Vipera berus*, which has two distinct colour morphs. Compared to the typical zigzag morph, melanistic individuals experienced thermal advantages (Forsman 1993), which contributed to increased body size (Luiselli 1993), reproductive success (Andren and Nilson 1981), and reproductive frequency (Capula and Luiselli 1994). Differential patterns of inter-morph survival between males and females has been shown, however, suggesting that fitness costs associated with colour differ by sex (Forsman 1995). Although these connections are not necessarily straightforward, overall, it is the magnitude of tradeoffs that will influence the maintenance of certain trait combinations and ultimately shape the evolution of phenotypes.

The general trend I have shown is that both colour morphs of *T .elegans* appear to exhibit similar achromatic and chromatic crypsis whereas the differences in colour and brightness conspicuousness between light *T. elegans* and *T. sirtalis* are more convincing. Although crypsis is greater for *T. sirtalis*, it may not necessarily be more effective in a general sense. In the research presented here, crypsis was measured when snakes were found lying motionless in the field and presumably basking — in other words, at one point in time and in one microhabitat type. The colour of *T. sirtalis* could represent a specialized colouration optimally suited for one habitat type whereas the colouration of light *T. elegans* could represent a compromised colouration that confers a high degree of crypsis in multiple habitat types (Merilaita et al. 1999). Snakes should therefore be followed closely over time and relocated on a regular basis to characterize potential colour-specific patterns of habitat use (Reinert 1994). Research could expand on previous habitat work (Charland and Gregory 1994) to quantify parameters such as snake and background colour as well as the frequency and visual complexity of habitat types (Houston et al. 2007). Ultimately, this more detailed study could elucidate temporal and spatial patterns of crypsis and determine if garter snake colouration represents a compromised or specialized strategy for crypsis.

Although evidence is weak, the overall trend suggests that *T. sirtalis* was more chromatically cryptic to the ferret visual model whereas light *T. elegans* was more cryptic to both bird models. What mechanisms could potentially influence differential crypsis between species that are found in the same habitat? We might expect more cryptic colouration in dark-coloured snakes if the selective pressure exerted by mammals was higher; that is, if mammalian predator density was higher or predator effectiveness was greater (Houston et al. 2007). Similarly, we might expect a comparable selective pressure if light-coloured snakes were easier to detect and hence more vulnerable to avian predators. In common collared lizards, *Crotaphytus collaris*, conspicuous sexual dichromatism between populations was hypothesized to be attributable to differences in predator types (Macedonia et al. 2002). Differential crypsis also occurs in the colour-changing lizard, the dwarf chameleon, *Bradypodion pumilum*, whereby individuals match background colours differently in response to either bird or snake predators (Stuart-Fox et al. 2008). Although garter snakes do not exhibit sexual dichromatism nor can they facultatively change colour, these examples suggest that differential crypsis is possible in response to differing selective pressures. There are, however, differences between these agents of selection in terms of predator strategy (use of vision vs. use of smell) and predator effectiveness (Lythgoe 1979, Gaffney and Hodos 2003), so these connections are not simply tied to differences in visual crypsis.

From the perspective of the human visual model, the results presented here were contrary to expectation. Although light *T. elegans* seem to be more difficult to detect in the field, the results suggest that light *T. elegans* were generally more conspicuous to humans both in terms of brightness and colour. Detection difficulty in this case could be more related to differences in visual processing rather than visual reception functions (i.e. conspicuousness of colour and brightness). In vertebrates, visual processing mechanisms contain receptive fields that are differentially sensitive to lines and gratings, which affect the perception of differences in colour and pattern composition (Bruce et al. 2003). For example, humans (Farmer and Taylor 1980) and birds (Dimitrova and Merilaita 2009) have difficulty in detecting objects as the visual complexity of backgrounds, independent of background matching, increases. Although light *T. elegans* and *T. sirtalis* were located in habitat types where background complexity was similar, differences in detection could perhaps be influenced by the complexity of dorsal colour patterns of snakes themselves – light *T. elegans* has a more complex dorsal pattern (i.e. stripes and blotches) than *T. sirtalis* or dark *T. elegans* (i.e. stripes only) – in the context of these habitats. It is becoming increasingly apparent that minimizing detection is not just a matter of background matching (Merilaita 2003, Dimitrova and Merilaita 2009) and future research needs to take into account both visual and cognitive processes (Bond 2007, Stevens 2007) in studying the evolution of animal colours.

In addition to colour, visual signals also consist of complex combinations of form (Cuthill et al. 2006, Lanridge 2006) and movement (Hailman 1977, Stevens 2007), which can influence detection. In Chapter 4, I demonstrated that light and dark *T. elegans* and *T. sirtalis* exhibited differences in behaviour when approached in the field as well as in performance; that is, light *T. elegans* were more likely to remain motionless when first detected and were generally slower, particularly at higher body temperatures. The tendency to either lie motionless or to move when detected as well as speed may contribute to differences in detection. Patterned animals may create a blurred image when moving and blend into the background as a result, referred to as the flicker-fusion effect (Pough 1976, Ruxton et al. 2004). If an animal moves faster than a predator's temporal acuity (i.e. ability to resolve differences between pattern elements), not only do patterns become difficult to distinguish but speed and direction of movement is also difficult to determine. To date, there is no strong experimental support for the flicker-fusion theory but it has been suggested that flicker-fusion might be more effective in low-light environments where the visual temporal acuity of animals is often reduced (Jarvis et al. 2002). Future work in this area should combine experimentation with the knowledge of visual image processing of predators (Bond 2007, Stevens 2007) using novel approaches, such as artificial neural networks (Merilaita 2007).

Animals simultaneously use two perceptual channels –achromatic and chromatic cues – to discriminate the brightness and colour in visual signals. It has been suggested that some animals may preferentially rely on one type of cue to detect objects, which presumably assists with the discrimination of food items and the detection of conspecifics. For example, domestic chicks relied largely on changes in surface luminance to detect prey objects (Osorio et al. 1999). In other work in lizards, the detection probability of anoline dewlaps increased when achromatic contrast was high

(Persons et al. 1999). Other animals, such as some groups of primates, used colour cues primarily for foraging tasks (Sumner and Mollon 2000), whereas moths and passerines have been shown to use both chromatic and achromatic cues in object detection (Kelber 2005, Stobbe et al. 2009). It may be that both achromatic and chromatic cues are important in prey detection but the relative importance of each at this point remains unknown. Currently, there is no general model that considers the relationship between both brightness and colour and the results presented here consider achromatic and chromatic crypsis separately. Consequently, the relative importance of differences in achromatic and chromatic crypsis observed between the different colour morphs and species is uncertain. If there is a potential interaction between achromatic and chromatic cues, and we are considering these data separately, this is a potentially important interaction that has yet to be explored.

In the research presented here, crypsis was measured from a similar proportion of males, nongravid and gravid females for dark and light *T. elegans* and *T. sirtalis*. Although I sampled an even number of both sexes and reproductive states between the colour morphs and species, gravid females were disproportionately represented in the total sample. This is not necessarily problematic because colour dimorphism does not occur between males and females nor does colour change with reproductive state in either morph of *T. elegans* or *T. sirtalis*. But, potential issues may arise if these groups either use microhabitats differently or are active at different times of the day. For example, individuals that are slower and therefore less likely to evade predation, such as gravid females (Shine 1980), or are less likely to survive a predation attempt, such as small juvenile snakes (Mushinsky and Miller 1993) may select habitats that are unfavourable for crypsis but favourable for other factors such as the number and proximity of retreat sites. Furthermore, Endler (1993) demonstrated that light quality varies with weather conditions, forest structure, and time of day, which can influence the appearance of animals in these environments. So, if small changes in habitat use are associated with either sex or reproductive state, then future research of this type should either focus on one group, or increase overall sample sizes to capture these potential differences. This way, the rigor of subsequent analyses could be increased by including sex and reproductive state as potential factors.

Background matching is typically evoked as a mechanism for animal crypsis. It is becoming increasingly apparent, however, that strategies other than background matching may be involved in minimizing animal conspicuousness (Schaefer and Stobbe 2006, Stevens 2007). For example, disruptive colouration operates by obscuring the true form of an animal through the use of strongly contrasting patterns to distract the attention of the viewer from the outline and contour of the animal (Cott 1940) and has been shown to minimize detection from predators (Merilaita 1998). Whether or not these mechanisms operate in an alternative or interrelated manner remains to be decided, but disruptive colouration has been shown to increase crypsis in natural (Merilaita 1998, Chiao and Hanlon 2001) and experimental systems (Cuthill et al. 2005, Merilaita and Lind 2005, Schaefer and Stobbe 2006). Although both *T. elegans* and *T. sirtalis* exhibit variation in colour patterns, identifying if and how pattern contributes to individual crypsis remains to be tested. Mechanisms by which crypsis is achieved are still unclear and future work should focus on the development of objective approaches to quantify spatial elements and novel ways to consider the contribution of both colour and pattern into studies of crypsis.

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CHAPTER 4

DOES APPEARANCE INFLUENCE BEHAVIOUR? EXPLORING INTERACTIONS BETWEEN DORSAL COLOURATION AND ANTIPREDATOR BEHAVIOUR IN GARTER SNAKES.

INTRODUCTION

In nature, organisms exhibit a wide variety of intra- and interspecific associations. For example, individuals interact through mutual co-existence, resource competition, parasitism, and predation, resulting in direct and indirect effects at the population and community levels (Abrams 1987). One type of association, predation, involves the consumption of one species by another and has been shown to reduce abundances (de Rivera et al. 2005), restrict species' distributions (Fortin et al. 2005), and ultimately influence the organization of communities (Paine 1969, Forsman et al. 2001). Predation is also a major selective force and, as a result, prey organisms often exhibit a diversity of life-history (Reznick and Endler 1982, Burks et al. 2000), behavioural (Bollens et al. 1992, Mathews et al. 2006), and morphological (Bergstrom and Reimchen 2003) adaptations to avoid being eaten.

Animals can use bright, contrasting colours to advertise their unpalatability or cryptic, concealing colours to minimize their detection in an effort to avoid predation (Edmunds 1974). Crypsis is considered an adaptation against visually-oriented predators whereby animals become less distinguishable from their background (see Chapter 3). Crypsis is a relative measure that depends on the background against which an animal is found and in some groups, such as frogs, immobility forms an integral part of this strategy (Heinen 1985, Morey 1990, Heinen and Hammond 1997, Cooper et al. 2008a). Animals that move not only compromise crypsis but can also potentially attract predators

(Gonka et al. 2000, Hoese et al. 2008, Ryer et al. 2008) and thereby should experience direct fitness-related consequences (Heinen and Hammond 1997, Vallin et al. 2006). For example, experimental work in aquatic insects demonstrated that larval chironomid prey species required both crypsis and immobility to avoid being preyed upon by stickleback fish, *Gasterosteus aculeatus* (Ioannou and Krause 2009). Given these direct implications for survival, we should therefore expect relationships to occur between the degree of an animal's crypsis and how it behaves in the wild.

Models of escape behaviour describe interactions between predators and prey and predict the optimal conditions under which prey should flee from a potential predator attack (Ydenberg and Dill 1986, Broom and Ruxton 2005). Early theoretical models suggested that the decision made by prey to flee vs. remaining motionless was influenced by the distance between them and predators (Heatwole 1968, Ydenberg and Dill 1986). Additional work has shown that escape behaviours may also depend on other factors such as predator search speed (Cooper 1997b), predator attack angle (Cooper et al. 2008b), and previous predator experience (Martin et al. 2009). Multiple factors can therefore influence antipredator behaviour (Stankowich and Blumstein 2005), but what function, if any, does an animal's appearance, such as colour, have in influencing behaviour, such as the tendency of snakes to remain still or to flee in the wild?

If prey have been detected by a predator and the decision to flee has been made, prey rely on secondary defense mechanisms, such as evasion, to elude predators (Jordao and Oliveira 2001, Gifford et al. 2008, Arendt 2009). We might expect tradeoffs with speed if animals depend primarily on being inconspicuous; for example, cryptic individuals might be slower because they rely mostly on avoiding detection, whereas conspicuous animals might be faster to compensate for increased visibility. Presumably, the relationship between colour pattern and antipredator behaviour may change depending on a variety of factors such as prey body temperature (Schieffelin and de Quieroz 1991, Passek and Gillingham 1997, Civantos et al. 2004), locomotory capacity (Martin et al. 2009), reproductive state (Shine et al. 2003a), and body size (Cuadrado et al. 2001). Because perceived risk increases substantially when prey are far from a potential refuge, we might also expect that proximity to shelter will modulate these relationships between colour and antipredator behaviours (Martin and Lopez 2000, Cooper 2003a, Stankowich and Blumstein 2005, Martin et al. 2009).

If evasion does not work and prey are captured, they have to rely on further tactics to avoid being killed, such as predator intimidation (Vallin et al. 2006), tonic immobility (Gregory and Gregory 2006, Gregory et al. 2007, Gregory 2008), or aggression (Crowley and Pietruszka 1983, Herzog and Burghardt 1986, Shine et al. 2003b). These direct interactions may result in death, but a prey sometimes escapes and injuries can be incurred. These non-lethal encounters with predators, including those resulting in injuries, can have longer-term consequences for individuals (Nakaoka 2000) that can ultimately manifest themselves as population-level effects (Lima 1998).

Scars found on animals, such as snakes, have been used to infer predation patterns (King 1987, Forsman 1995). Drawing relationships between injuries and predation can be problematic (Schoener 1979); for example, scars may occur occasionally because of non-predation related activities (Vitt et al. 1974) and even when scars are due to attempted predation, they may be more frequent on larger animals because smaller animals are more likely to be killed out-right rather than sustain an injury. In addition, larger/older animals may exhibit more injuries simply because they have had a longer period of time over which to accumulate scars (Mushinsky and Miller 1993, Gregory and Isaac 2005). In the absence of long-term mark recapture studies (Brodie 1992), we are unable to say much about characteristics of snakes that have died and what, if any, was the potential role of previous injury or other factors, such as colour. It is nonetheless plausible that animals vary in their susceptibility to injury because of other characteristics such as degree of conspicuousness. Here, I use data on injuries in snakes to begin testing associations between injuries and snake colour. The results of these tests will provide the basis for hypotheses for future tests about the fitness-related consequences of these traits (Tordoff 1980, Sandoval 1994, Heinen and Hammond 1997, Civantos et al. 2004, Croshaw 2005, Palleroni et al. 2005).

In a previous chapter (Chapter 3), I demonstrated that there were no differences in crypsis in terms of colour and brightness (i.e. chromatic and achromatic crypsis) between the light and dark colour morphs of Western Terrestrial Garter Snakes, *Thamnophis elegans*, from the perspective of mammal and bird predators. Light *T. elegans*, on the other hand, was significantly less achromatically cryptic to all three visual models than co-occurring Common Garter Snakes, *T. sirtalis*. In terms of colour, light *T. elegans* was less cryptic, albeit not significantly, than *T. sirtalis*. Also, the dark-coloured snakes — dark *T. elegans* and *T. sirtalis* — were significantly more cryptic to humans both in terms of colour whereas differences in achromatic crypsis varied between light and dark snakes.

Here, I investigate potential associations among snake crypsis, behaviour, and injury rate, as quantified in the laboratory and observed in the field. More specifically, I test relationships between colour and behaviour assessed before and after snake capture, crawling performance, and incidence of injuries. I ask the following five questions:

1. Are there tradeoffs between snake crypsis and distance to shelter?

Because crypsis was similar between light and dark *T. elegans*, I predicted that both colour morphs would be found at similar distances from refuge. In addition, I predicted that light *T. elegans* would be found closer to refuge than the more cryptic *T. sirtalis* because they were more visible to predators and therefore would incur more risks when far from refuge.

2. Do less cryptic snakes flee sooner from a threat?

Because crypsis is presumably maximized when individuals remain motionless, I predicted that snakes that were more cryptic would remain motionless as long as possible preceding capture in an attempt to maximize their crypsis. Hence, I predicted no difference in pre-capture behaviour between dark and light *T. elegans*. Less cryptic snakes, however, would initiate their escape earlier as they were approached and I predicted light *T. elegans* would initiate their retreat earlier than *T. sirtalis*. Also, I predicted that snakes that remained motionless longer in the field (that is, between detection and capture) would be the more cryptic types because crypsis was being relied upon right up to the last moment before capture.

3. Are less cryptic snakes faster?

I predicted that snakes that were more cryptic would be less reliant on speed to evade predators and therefore be slower. I therefore predicted that there would be no speed difference between the two colour morphs of *T. elegans* whereas *T. sirtalis* would be slower.

4. Do post-capture behaviours vary with snake crypsis?

If snakes are caught by a predator, presumably all individuals, regardless of crypsis, would employ post-capture behaviours to free themselves. I therefore predicted that no differences in post-capture behaviours would be associated with snake crypsis.

5. Are more cryptic snakes less likely to be injured?

I predicted that more cryptic snakes would exhibit fewer injuries than less cryptic snakes because of their lower probability of detection by a predator. I therefore predicted no difference in injuries between the two colour morphs of *T. elegans*, whereas in the interspecific comparison, light *T. elegans* would exhibit more injuries.

METHODS

Study area, snake capture & measurements

I undertook field work between 2005-2008 at three study sites in British Columbia (BC), Canada where the light and dark morphs of the Western Terrestrial Garter Snake, *Thamnophis elegans* and the Common Garter Snake, *T. sirtalis*, occur (see Chapter 1, Fig. 1). I searched for snakes at the three sites on foot and captured individuals by hand. Body (cloacal) temperature (T_b) was taken immediately after capture using a quick-reading digital thermometer (HI 9063 thermometer, Hanna Instruments, Singapore). I held snakes by the midsection and the tail in order to minimize temperature change attributable to my handling.

I then held snakes in the air by the tail for 30 seconds to simulate a generalized predatory attack (snakes presumably are frequently grasped by the tail by predators, as loss of part of the tail is common). During this time, I noted if snakes hung motionless or if they moved either by twirling, thrashing their body downwards, or general struggling. I recorded the distance of each snake to cover, snake behaviour when detected (i.e. motionless or moving) and immediately prior to capture (i.e. for snakes that were motionless when detected, whether they remained motionless or started moving).

I measured snout-vent length (SVL), determined the sex of each snake, and assessed each adult female's reproductive condition by gently palpating it in the abdomen to determine whether it was gravid. As these measurements were being collected, I placed a mercury thermometer on the ground at each capture site (for at least 10 minutes to allow equilibration) to measure operative temperature (Bakken 1992). Next, I recorded the presence of injuries under the working hypothesis that scars or stump tails resulted from failed predation attempts. Following this, I individually marked each snake by clipping a unique combination of subcaudal scutes on the underside of the tail for future identification. To avoid pseudoreplication, I used only original captures in statistical analyses.

Analyses

The relationships investigated here take body size into account because numerous functions are linked with body size in snakes (Stevenson 1985, Finkler and Claussen 1999). For example, small and large snakes differ in speed and also presumably in vulnerability, so we might expect antipredator behaviours to differ accordingly. Also, the probability of having been injured should increase with age, which is roughly correlated with size, so bigger snakes should be more likely to have acquired injuries than small snakes, even in the absence of differential vulnerability to predators (Gregory and Isaac 2005).

For all field captures, I used ANCOVA to test for relationships between the distances snakes were from refuge in the field and body size for light and dark colour morphs of *T. elegans* and for light *T. elegans* and *T. sirtalis*. Other potentially important contributing factors that were included in the analyses were T_b, sex/reproductive condition (i.e. male, nongravid female, or gravid female) and injury, which can be considered a proxy for previous predator experience. Distance to cover was treated as the independent variable and body size (SVL) and T_b were treated as covariates. I included interaction terms to test for heterogeneity of slopes (e.g. SVL X morph/species, T_b X morph/species, etc.) and removed all nonsignificant interaction terms to simplify the analyses.

I used logistic regression to determine factors that best predicted the probability of finding a snake either moving or lying still. Independent variables included colour (light/dark morph of *T. elegans* or light *T. elegans*/*T. sirtalis*), sex/reproductive state, body size (SVL, mm), distance to refuge, injury, and appropriate interaction terms. For snakes that were not moving when first seen, I used logistic regression to test the effect of these same factors on the behaviour (moved or did not move) of these snakes before capture. For post-capture behaviour, I used chi-square analyses to compare the proportions of observed frequencies of snakes that either hung limp and remained motionless or squirmed, twirled, or thrashed by colour morph (light/dark *T. elegans*) and species (light *T. elegans* vs. *T. sirtalis)*. I used logistic regression again to test the influence of body size and colour on the occurrence of injury using the same factors described above.

Performance

I tested the potential relationship between crawling speed and body colouration in the laboratory using male and nongravid light and dark *T. elegans* and *T. sirtalis* caught in 2006 and 2007. The experiments were repeated on three separate occasions – June and August 2006, and August 2007, using different animals but with the same experimental design. I constructed a 1.0-m long, 0.5-m wide, and 0.5-m high racetrack out of cardboard and positioned it on a laboratory bench. A dark pillowcase was placed at the end of the racetrack as a target for shelter. To measure crawling speeds, I chased snakes down the racetrack and continuously tapped their tails using a paintbrush to stimulate movement. I measured each trial using a digital stopwatch and then converted each time to speed (m/sec). Because crawling speed varies with body size (Finkler and Claussen 1999), I divided each speed by SVL (mm) to obtain relative speed (m/sec/mm).

The performance of various physiological and behavioural functions is positively correlated with increasing body temperature in snakes (Stevenson et al. 1985), so I tested each individual snake at 6 different T_b ranges: 15-16°C, 21-22°C, 24-25°C, 27-28°C, 31-32°C, and 35-36°C. In each experiment, I controlled T_b by placing each animal in either a constant-temperature cooler or incubator approximately 1 h before each trial. Before each snake was tested, I confirmed T_b by measuring cloacal temperature with an Omega Digital Thermometer (Model: HH82; Omega Engineering Stamford, CT).

Snakes were deprived of food five days prior to racing to ensure that their speed was not hindered by the weight of food (Garland and Arnold 1983). Snakes were also given at least a 1-h rest between trials. I randomized the order in which individuals were tested at a given temperature as well as the order in which I ran trials at the different temperatures. Crawling speed was measured three times for each individual and if a snake balked while crawling by either refusing to move down the track or by reversing direction, the trial was stopped and immediately restarted (within 10 s). If this behaviour was repeated, the snake was taken out of rotation and re-run at the end of the trial. Speed trials were used for the crawling experiment only if at least two of the three trials at a particular T_b were completed (hence smaller sample sizes in some cases). Once the experiments were completed, snakes were fed and released under favourable weather conditions at their point of capture.

I tested differences in mean crawling speed using all three crawling trials for each snake at each temperature (Isaac and Gregory 2005). Because speeds were measured repeatedly for the same individual over different temperatures, the data were not statistically independent. I therefore used univariate nested analysis of variance (ANOVA) models with individuals nested within colour morph/species to compare mean crawling speed between individuals, sexes, colour morphs/species, and temperature levels. To avoid inflation of overall α-error in comparisons of crawling speed at different T_bs, I limited inter-temperature comparisons to orthogonal (i.e. independent) tests between adjacent temperatures (e.g. 15-16 °C to 21-22 °C, 21-22°C to 25-26 °C, etc.).

All statistical tests and graphing were performed using SAS 9.2. Post-hoc model comparisons were performed using the Tukey-Kramer method and I assessed significance at $\alpha = 0.05$. All animal maintenance and experimental procedures complied with guidelines for live reptiles and were approved by the University of Victoria Animal Care Committee.

RESULTS

Cover

In the intraspecific comparison, both colour morphs of Western Terrestrial Garter Snakes, *Thamnophis elegans,* were found at similar distances from cover ($F_{1,\ 41} = 0.02$, $p = 0.8924$). This leant support to the original prediction that equally cryptic snakes would be of similar distances to refuge. There was also no influence of body size (SVL; $F_{1,\ 41} = 0.17$, $p = 0.6785$), body temperature (T_b; $F_{1,\ 41} = 1.50$, $p = 0.2274$), nor sex/reproductive state in this relationship ($F_{1,\ 41} = 2.23$, $p = 0.1428$). Contrary to expectation, light *T. elegans* and Common Garter Snakes, *T. sirtalis*, were also found at comparable distances from shelter ($F_{1,\ 32}= 0.76$, $p = 0.3902$). Body size (SVL; $F_{1,\ 32} = 0.04$, $p = 0.8509$), body temperature (T_b; $F_{1,\ 32} = 51$, $p = 0.4806$), or sex ($F_{1,\ 32} = 0.04$, $p = 0.8439$) also did not influence the proximity of light and dark snakes to cover.

Behaviour when detected

Gravid and nongravid females exhibited similar behaviours when first detected in the field, therefore, data for these groups were combined for both colour morphs of *T. elegans* (Wald's $\chi^2_1 = 0.7578$, $p = 0.3840$) and light *T. elegans* and *T. sirtalis* (Wald's $\chi^2_1 = 1.6184$, $p = 0.2033$). I then tested the effect of sex and found that it did not significantly influence the probability of a snake moving when first detected for either the intraspecific (Wald's $\chi^2_1 = 0.1034$, $p = 0.7478$) or interspecific comparison (Wald's $\chi^2_1 = 1.9873$, $p = 0.1586$); males and all females were therefore combined. All two- and three-way interactions (e.g. distance-to-cover X colour, etc.) were non-significant and therefore removed to simplify analyses.

The probability that a snake was moving when first detected in the field was significantly higher for light *T. elegans* (Wald's $\chi^2_1 = 6.3153$, $p = 0.0120$) than for the dark morph. This result was contrary to the expectation of the equally cryptic morphs exhibiting comparable pre-capture behaviour. In addition, snakes found farther away from cover were significantly more likely to be moving when first seen (Wald's $\chi^2_1 = 5.3367$, $p = 0.0209$; Fig. 1a). Small snakes were significantly more likely to be moving when seen (Wald's $\chi^2_1 = 5.9014$, $p = 0.0151$). Snakes that exhibited some type of injury were more likely to be moving when first detected (Wald's $\chi^2_1 = 3.4242$, $p = 0.0642$), suggesting that perhaps previous predator exposure influenced individual behaviour.

In the interspecific comparison, there was no difference between light *T. elegans* and *T. sirtalis* in the likelihood of these species moving when first seen (Wald's $\chi^2_1 = 1.1425$, $p = 0.2851$). This result was contrary to the expectation that the more cryptic *T. sirtalis* would be more likely to be motionless when detected in the field. Overall, snakes found farther from cover were also more likely, with marginal significance (Wald's $\chi^2_1 = 3.0765$, $p = 0.0794$), to be moving when first detected (Fig. 1b). Small light *T. elegans* and small *T. sirtalis* were also significantly more likely to be moving (Wald's $\chi^2_1 = 5.4820$, $p = 0.0192$). Unlike the intraspecific comparison, there was no influence of injuries on behaviour when first seen in the field (Wald's $\chi^2_1 = 0.2135$, $p = 0.6441$).

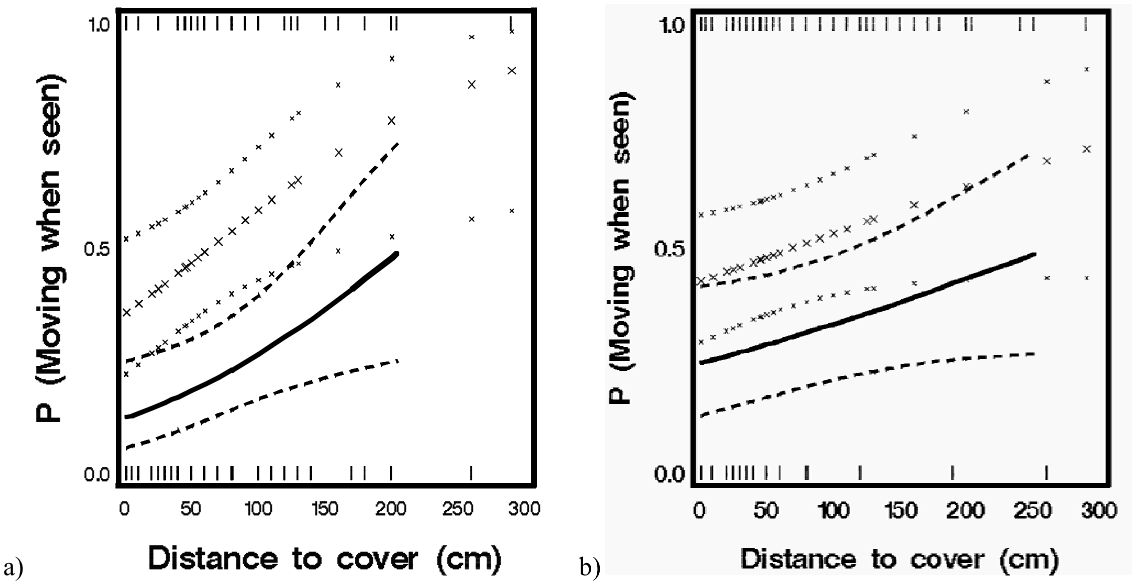

Figure 1. Probability of snakes either moving when seen in the field (1.0) or lying still (0.0) against distance to cover (cm) for a) light and dark Western Terrestrial Garter Snakes, *Thamnophis elegans*, and b) light *T. elegans* and Common Garter Snakes, *T. sirtalis*. Light *T. elegans* had a higher probability of moving when first detected in the intraspecific comparison (a). Overall, snakes found farther from cover were more likely to be found moving, in both comparisons. Light *T. elegans* are represented by 'X's and surrounded by 95% confidence limits in a) and b). The solid black line represents the predicted values from the logistic regression and is surrounded by segmented lines representing 95% confidence limits for dark *T. elegans* in a) and *T. sirtalis* in b). The actual data points are indicated at the top and bottom of the graph.

Behaviour of motionless snakes before capture

Gravid and nongravid females that were not moving when first seen exhibited similar behaviour when approached in the field (intraspecific comparison: Wald's $\chi^2_1 = 0.2054$, p = 0.6504; interspecific comparison: Wald's $\chi^2_1 = 0.0069$, p = 0.9336) and so these data were grouped together. There was no difference in the probability that males and females moved when approached in the field for either the intraspecific (Wald's $\chi^2_1 = 2.6939$, p = 0.1007) or interspecific comparison (Wald's $\chi^2_1 = 0.0513$, p = 0.8209); sexes were therefore combined. All two- and three-way interactions (e.g. size X colour, sex X size X colour, etc.) were non-significant and therefore removed.

Of the motionless snakes that I detected in the field, some remained in place until I touched them, whereas others moved off quickly as I approached. Both light and dark *T. elegans* had an equal probability of moving before capture (Wald's $\chi^2_1 = 0.2224$, p =

0.6372; Fig. 2a). There was also no effect of body size on the probability that snakes moved as I approached (Wald's χ^2_1 = 0.0243, p = 0.8760). Snakes found farther from cover had a significantly higher probability of moving when approached (Wald's χ^2_1 = 0.2714, p = 0.0462). Snakes that exhibited some type of injury were more likely, although evidence is weak, to remain motionless up to the point of capture (Wald's χ^2_1 = 2.9661, p = 0.0850).

In the interspecific comparison, neither species (Wald's χ^2_1 = 0.2287, p = 0.6325) nor body size (Wald's χ^2_1 = 1.7385, p = 0.1873) were significant in explaining the probability of snakes moving before capture (Fig. 2b). This result was contrary to the expectation that the more cryptic *T. sirtalis* would be more likely to be motionless right up to the point of capture. Both light *T. elegans* and *T. sirtalis* found farther from cover were, however, significantly more likely to move before capture (Wald's χ^2_1 = 3.5755, p = 0.0586), however, injuries had no influence on the tendency of snakes to flee (Wald's χ^2_1 = 0.1186, p = 0.7305).

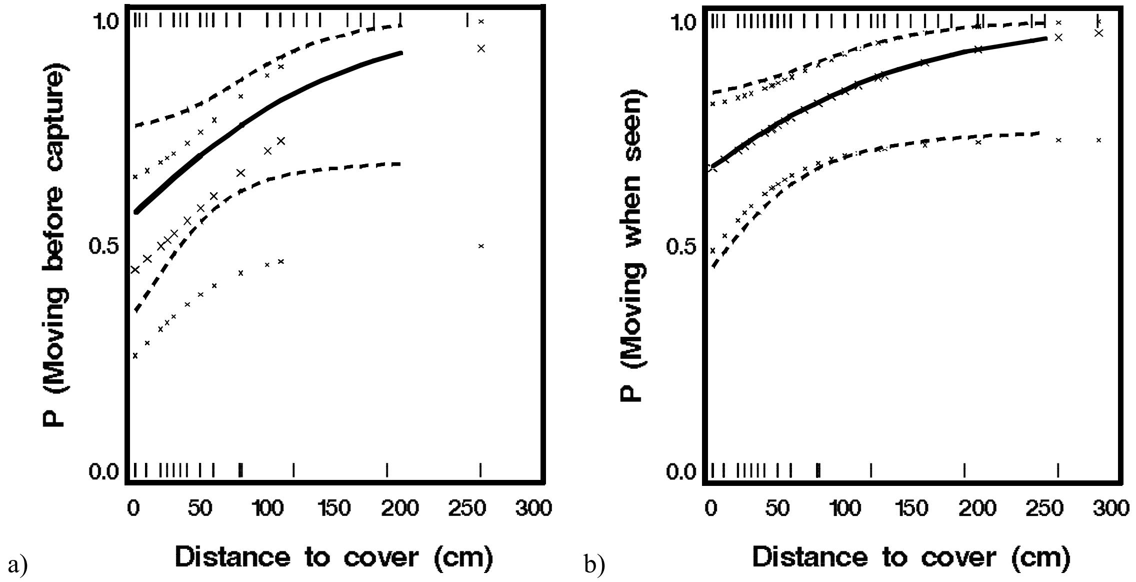

Figure 2. Probability of a snake that was originally detected lying still, either moving (1.0) or remaining motionless (0.0) before capture vs. distance to cover (cm) in a) light and dark Western Terrestrial Garter Snakes, *Thamnophis elegans*, and b) light *T. elegans* and Common Garter Snakes, *T. sirtalis*. Snakes used in this analysis are a subset (i.e. motionless when first detected in the field) of snakes from Figure 1. The probability of a snake moving before capture did not differ between colour morphs, between species, or between snakes of different size. Snakes were, however, significantly more likely to move before capture if they were found far from refuge. Light *T. elegans* are represented by 'X's and surrounded by 95% confidence limits in a) and b). The solid black line represents the predicted values from the logistic regression and is surrounded by segmented lines representing 95% confidence limits for dark *T. elegans* in a) and *T. sirtalis* in b). The actual data points are indicated at the top and bottom of the graph.

Performance

Overall, males were significantly faster than nongravid females (light vs. dark *T. elegans*: $F_{1,1030} = 60.85$, $p < 0.0001$; light *T. elegans* vs. *T. sirtalis*: $F_{1,1531} = 49.35$, $p < 0.0001$) and thus, all analyses were divided by sex (Tables 1 & 2). As expected, there was significant inter-individual variability in performance ($p < 0.0001$ in all comparisons). Also, snakes became faster with increasing T_b as was predicted (Fig. 3 & 4).

Dark *T. elegans* were significantly faster than light *T. elegans*, although this difference varied with T_b (i.e. significant interaction between colour morph and T_b; Table 1). In males, light *T. elegans* were significantly faster at the lowest T_b, 15-16ºC, whereas dark *T. elegans* were significantly faster at a range of higher T_bs including 27-28°C, 31-32°C, and 35-36°C (Fig. 3a). In females, light *T. elegans* were significantly faster at 24-25ºC, whereas dark *T. elegans* were significantly faster at 31-32ºC and 35-36ºC (Fig. 3b). Overall, dark *T. elegans* of both sexes were generally faster at the higher T_bs.

Table 1. ANOVA comparing locomotory performance as measured by relative crawling speed (m/sec/mm) between dark and light Western Terrestrial Garter Snakes, *Thamnophis elegans,* for males and females. Overall, dark *T. elegans* were faster than light *T. elegans* but these differences varied with body temperature.

Dependent variable	**Factor**	**F-value, $_{df}$, p – value**
Light *T. elegans* vs. dark *T. elegans* – Males		
Relative Speed (m/sec/mm)	Colour	$F_{1,456} = 34.63$, $p < 0.0001$
	Temperature	$F_{5,456} = 78.93$, $p < 0.0001$
	Individual (colour)	$F_{26,456} = 12.44$, $p < 0.0001$
	colour X temperature	$F_{5,456} = 8.02$, $p < 0.0001$
Light *T. elegans* vs. dark *T. elegans* – Females		
Relative Speed (m/sec/mm)	Colour	$F_{1,574} = 8.36$, $p = 0.0040$
	Temperature	$F_{5,574} = 115.77$, $p < 0.0001$
	Individual (colour)	$F_{34,574} = 20.34$, $p < 0.0001$
	colour X temperature	$F_{5,574} = 9.77$, $p < 0.0001$

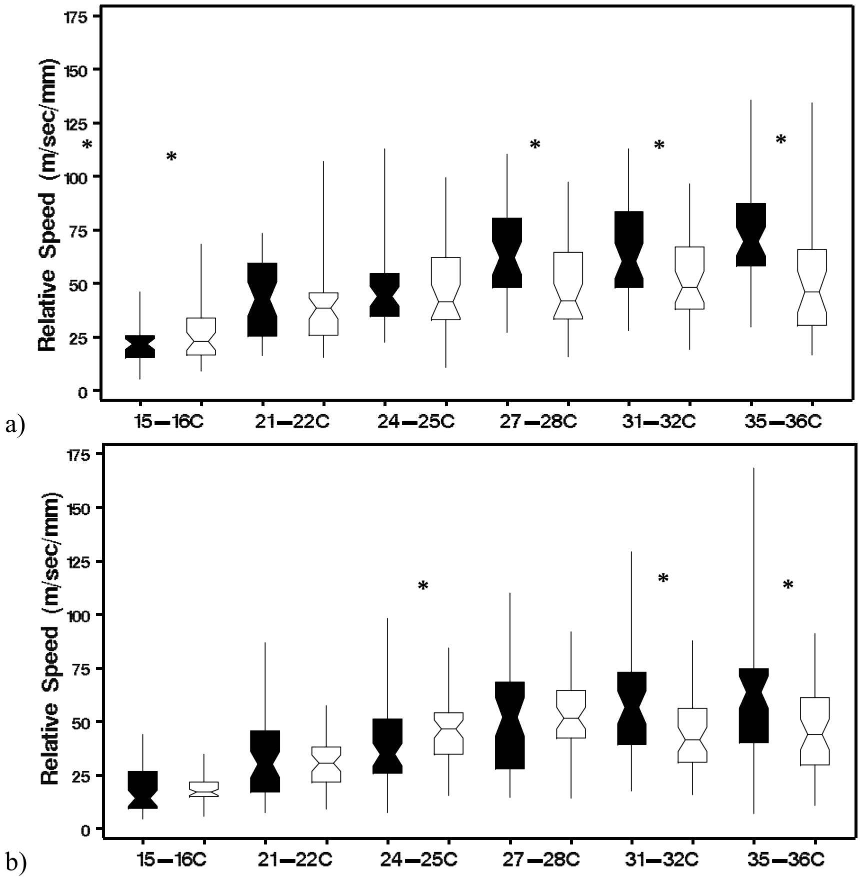

Figure 3. Relative crawling speed (m/sec/mm) for a) male and b) female dark and light Western Terrestrial Garter Snakes, *Thamnophis elegans*, at different body temperatures (T_bs). Overall, dark *T. elegans* were faster across the range of T_bs, however, light male *T. elegans* were significantly faster at 15-16ºC and light female *T. elegans* were significantly faster at 24-25ºC. Significant differences in relative speed are indicated by '*'. Relative speed was calculated by dividing crawling speed by SVL to adjust for differences related to body size and multiplied by 10000 to aid interpretation. Light *T. elegans* are indicated by white boxes and dark *T. elegans* are indicated by black boxes. Each box represents 50% of the data, the lines extend to the minimum and maximum value, and the median is indicated by the notches.

Overall, the crawling performance of light *T. elegans* was faster than *T. sirtalis* for males, but differences in performance varied with T_b (i.e. significant interaction term; Table 2). For example, male light *T. elegans* were significantly faster across most T_bs (15-16ºC, 21-22ºC, 25-26ºC and 35-36ºC; Fig. 4a). In females, *T. sirtalis* were faster than light *T. elegans* but again, the strength of difference in performance varied with T_b; that is, female *T. sirtalis* were significantly faster than light *T. elegans* at 15-16ºC, 27-28ºC and 31-32ºC (Fig. 4b).

Table 2. ANOVA comparing locomotory performance as measured by relative speed (m/sec/mm) between light Western Terrestrial Garter Snakes, *Thamnophis elegans*, and Common Garter Snakes, *T. sirtalis* for males and females. Overall, male light *T. elegans* were faster at most T_bs, whereas female *T. sirtalis* were significantly faster, but these differences varied with body temperature.

Dependent variable	**Factor**	**F-value, $_{df}$, p – value**
Light *T. elegans* vs. *T. sirtalis* – Males		
Relative Speed (m/sec/mm)	Species	$F_{1,776} = 2.44$, $p = 0.1189$
	Temperature	$F_{5,776} = 156.99$, $p < 0.0001$
	Individual (species)	$F_{47,776} = 11.91$, $p < 0.0001$
	Species X temperature	$F_{5,776} = 2.51$, $p = 0.0289$
Light *T. elegans* vs. *T. sirtalis* – Females		
Relative Speed (m/sec/mm)	Species	$F_{1,755} = 16.14$, $p < 0.0001$
	Temperature	$F_{5,755} = 149.26$, $p < 0.0001$
	Individual (species)	$F_{45,755} = 19.45$, $p < 0.0001$
	Species X temperature	$F_{5,755} = 2.68$, $p = 0.0208$

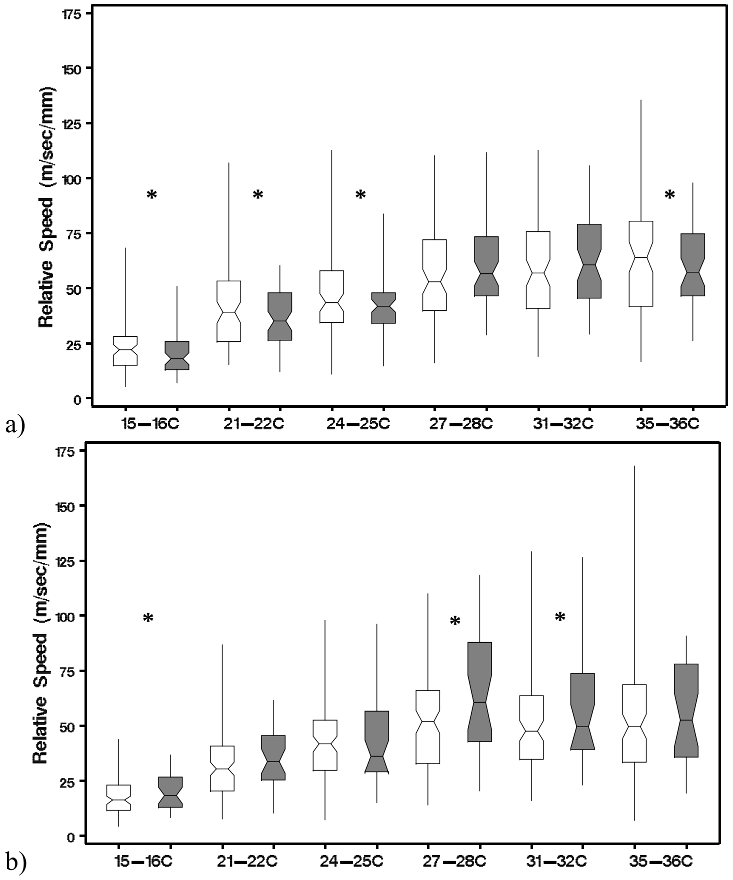

Figure 4. Relative crawling speed (m/sec/mm) for a) male and b) female light Western Terrestrial Garter Snakes, *Thamnophis elegans,* and Common Garter Snakes, *T. sirtalis,* at six different body temperatures (T_bs). In males, light *T. elegans* were faster overall, whereas in females, *T. sirtalis* were faster, however, these differences varied with T_bs. Significant differences are indicated by '*'. Relative speed was calculated by dividing crawling speed by SVL and multiplied by 10000 to aid interpretation. Light *T. elegans* are indicated by white boxes and *T. sirtalis* are indicated by grey boxes. Each box represents 50% of the data, the lines extend to the minimum and maximum value, and the median is indicated by the notches.

Behaviour after capture

In general, the majority of dark and light *T. elegans* thrashed or twirled their bodies when exposed to 'simulated' predatory attacks (Fig. 5a). This proportion of behaviour was consistent between colour morphs ($\chi^2_1 = 1.0115$, $p = 0.3145$). There was, however, a significant difference between the two species overall in the proportion of snakes that either moved or hung limp when suspended by the tail (Fig. 5b). Significantly fewer *T. sirtalis* exhibited body movement after capture compared to light *T. elegans* ($\chi^2_1 = 9.6624$, $p = 0.0019$).

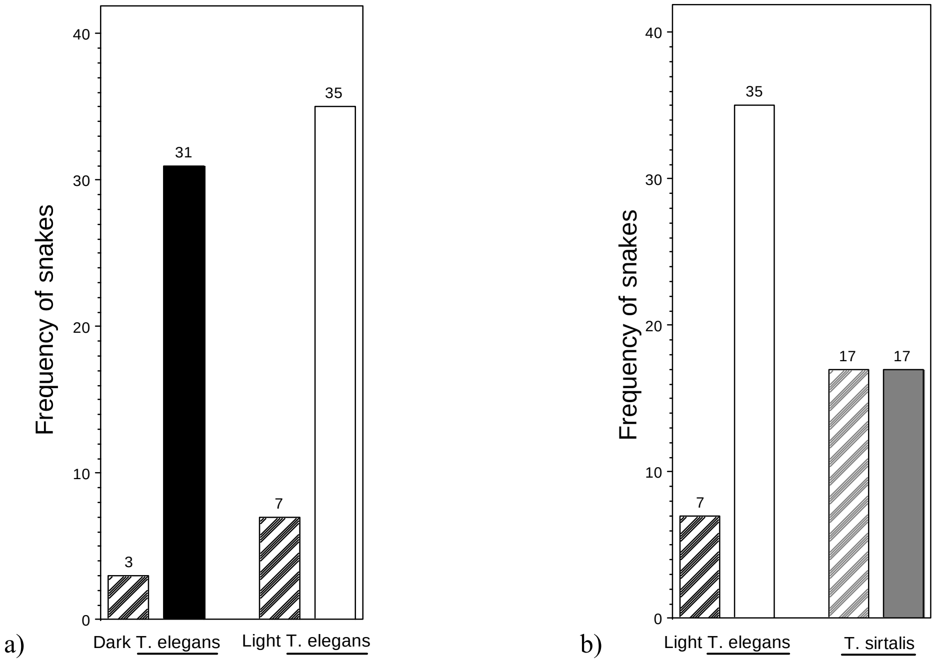

Figure 5. Frequency of snakes that hung motionless (hatched bars) or displayed movement (solid bars) when held by the tail following capture for a) dark (black bars) and light (white bars) Western Terrestrial Garter Snakes, *Thamnophis elegans,* and b) light *T. elegans* (white bars) and Common Garter Snakes, *T. sirtalis* (grey bars). Overall, a large number *T. elegans* of both colour morphs exhibited movement when captured and the proportion of snakes that hung limp or moved was consistent between colour morphs. In the interspecific comparison; however, more *T. sirtalis* hung motionless when held in the hand.

When these groups were analysed separately by sex and reproductive state, considerably different patterns emerged between these groups. In the intraspecific comparison, all gravid females, regardless of colour morph, and most males ($\chi^2_1 =$

0.5742, p = 0.4486) displayed some type of movement when suspended in the air by the tail (Fig. 6a & c). There was a significantly different pattern for nongravid females; all dark *T. elegans* moved, whereas behaviours were more variable for the light morph (χ^2_1 = 6.111, p = 0.0134; Fig. 6b).

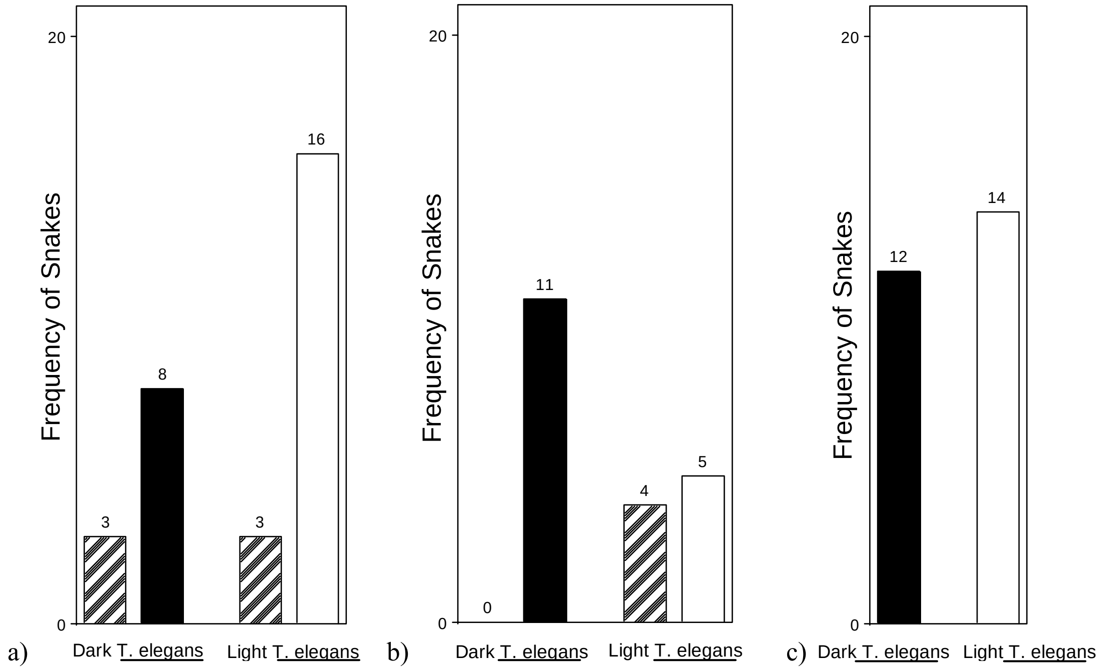

Figure 6. Frequency of snakes that hung motionless (hatched bars) or displayed movement (solid bars) when held by the tail following capture for a) males, b) nongravid, and c) gravid females in dark (black bars) and light (white bars) Western Terrestrial Garter Snakes, *Thamnophis elegans*. All gravid females of both colour morphs and nongravid dark *T. elegans* exhibited movement when held by the tail, however, the frequency of movement behaviours exhibited by nongravid light *T. elegans* and males were more variable.

In the interspecific comparison, males showed significantly disparate patterns; more light *T. elegans* exhibited movement, whereas more *T. sirtalis* hung limp when exposed to the ‘simulated’ predator attack (χ^2_1 = 9.0337, p = 0.0027; Fig. 7a). No post-capture behaviour differences emerged for nongravid females (χ^2_1 = 0.2338, p = 0.6287; Fig. 7b). And finally, there was a significant difference in frequency of movemet behaviours exhibited by gravid females; all light *T. elegans* exhibited post-capture behaviour whereas *T. sirtalis* behaviours were more variable (Fig. 7c).

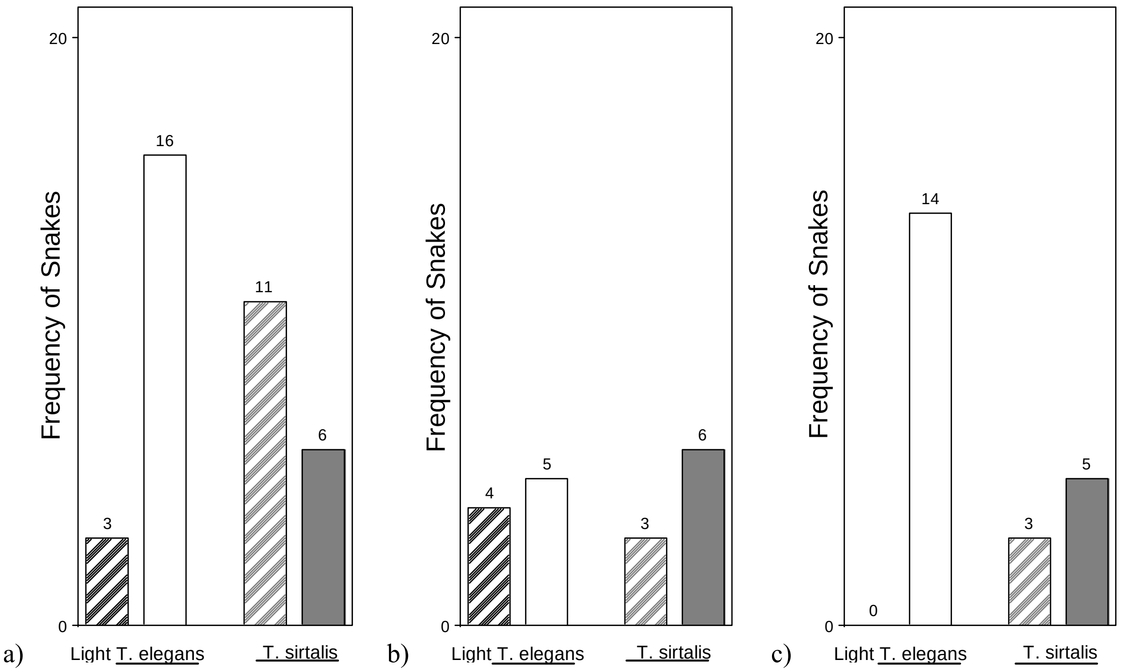

Figure 7. Frequency of snakes that hung motionless (hatched bars) or displayed movement (solid bars) when held by the tail following capture for a) males, b) nongravid, and c) gravid females in light Western Terrestrial Garter Snakes, *Thamnophis elegans* (white bars), and Common Garter Snakes, *T. sirtalis* (grey bars). Gravid females and most light *T. elegans* males exhibited movement when held by the tail, however, the frequency of movement behaviours exhibited by all groups of *T. sirtalis* were more variable.

Injury rates

In the intra-specific comparison, the probability of snakes having an injury was significantly different for males and females (sex: Wald's $\chi^2_1 = 8.2588$, $p = 0.0041$), so data were divided and analysed separately. In males, there was a significant difference in the likelihood of light and dark *T. elegans* having some type of injury (Wald's $\chi^2_1 = 8.3041$, $p = 0.0040$); however, this relationship varied with body size (SVL*colour: Wald's $\chi^2_1 = 10.9419$, $p = 0.0009$). In males, large light *T. elegans* and small dark *T. elegans* were more likely to have an injury (Fig. 8a). In females, light *T. elegans* had a significant higher probability of having an injury than dark *T. elegans* (Wald's $\chi^2_1 = 4.7429$, $p = 0.0294$). Also, overall, large snakes of both colour morphs were more likely to have an injury than smaller snakes (Wald's $\chi^2_1 = 28.5561$, $p < 0.0001$; Fig. 8b).

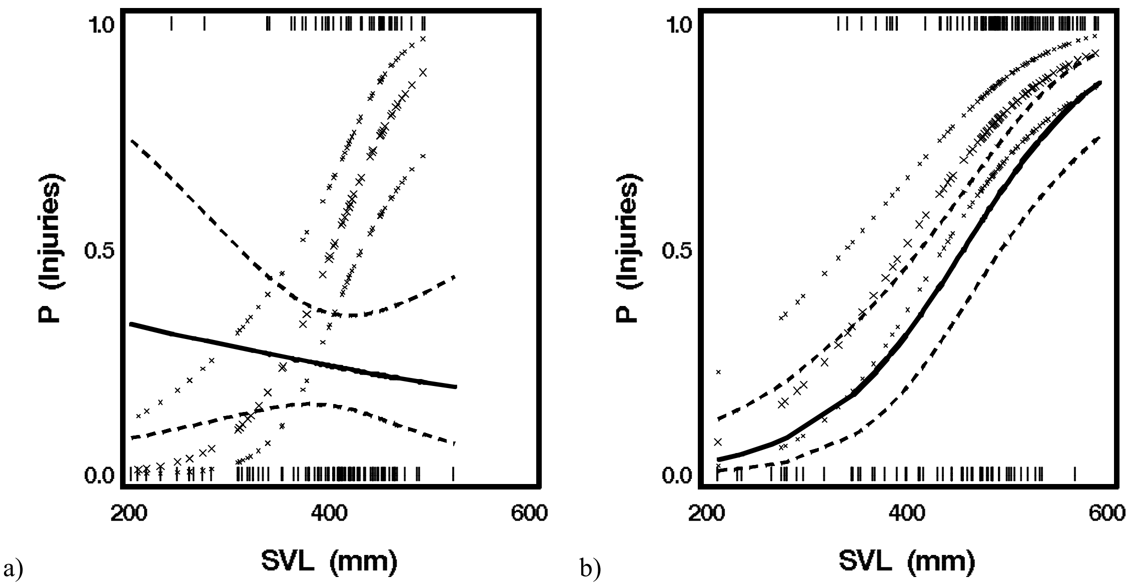

Figure 8. Probability of a snake being injured vs. body size (SVL, mm) for a) male and b) female Western Terrestrial Garter Snakes, *Thamnophis elegans,* of both colour morphs. In males, larger light and smaller dark *T. elegans* had a significantly higher probability of being injured. In females, light *T. elegans* had a significantly higher probability of being injured and overall, the probability of being injured increased with female snake body size. Light *T. elegans* are represented by 'X's and surrounded by 95% confidence limits in a) and b). The solid black line represents the predicted values from the logistic regression and is surrounded by segmented lines representing 95% confidence limits for dark *T. elegans* in a) and b). The actual data points are indicated at the top and bottom of the graph.

In the interspecific comparison, there was no significant difference in the probability of a snake being injured between males and females (Wald's $\chi^2_1 = 0.0436$, $p = 0.8346$) and so the data were combined. All non-significant 2-way and 3-way interactions (size X colour, etc.) were removed from the analysis. Light *T. elegans* were more likely, although not significantly, to have an injury than *T. sirtalis* (Wald's $\chi^2_1 = 3.1591$, $p = 0.0755$). Also, the probability of both species being injured increased with body size (Wald's $\chi^2_1 = 48.7576$, $p < 0.0001$; Figure 9).

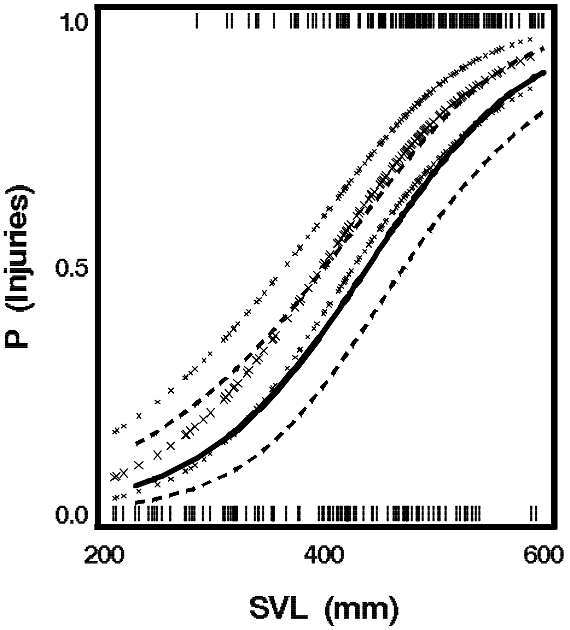

Figure 9. Probability of a snake being injured vs. body size (SVL, mm) for light Western Terrestrial Garter Snakes, *Thamnophis elegans*, and Common Garter Snakes, *T. sirtalis*. Light *T. elegans* had a higher probability of being injured, however, this difference was not significant. Also, larger snakes of both species had a higher probability of being injured. Light *T. elegans* are represented by 'X's and surrounded by 95% confidence limits. The solid black line represents the predicted values from the logistic regression and is surrounded by segmented lines representing 95% confidence limits for *T. sirtalis.* The actual data points are indicated at the top and bottom of the graph.

DISCUSSION

There is an increasing body of evidence demonstrating the link between crypsis and behaviour in various taxa, including anurans (Heinen 1985, Morey 1990, Heinen and Hammond 1997, Croshaw 2005, Cooper et al. 2008b) and reptiles (Heatwole 1968, Jackson et al. 1976, Martin and Lopez 2000, Cuadrado et al. 2001, Carretero et al. 2006). Although these studies yield interesting insights into animal behaviour and the potential tradeoffs that may occur between behaviour and crypsis, what they generally lack is the evaluation of crypsis from the perspective of relevant viewers, such as predators, and a demonstration of how colour and behaviour may serve an adaptive function that contributes to fitness. The research presented in this chapter focuses on the comparison of snake behaviour, performance, and the probability of injury between colour morphs and species in the context of snake crypsis as perceived by bird and mammal predators.

In general, crypsis was similar between both colour morphs of Western Terrestrial Garter Snakes, *Thamnophis elegans,* whereas Common Garter Snakes, *T. sirtalis,* were more cryptic than light *T. elegans.* I predicted that more conspicuous snakes would be found closer to refuge because the perception of risk associated with increased visibility would be minimized (Stankowich and Blumstein 2005). Alternatively, cryptic animals might be found at a variety of distances and not necessarily close to cover because visibility and susceptibility to predators would be reduced. As it turns out, there were no discernible relationships between body size and distance to cover for either colour morphs or species. Similarity in crypsis perhaps explained why no differences emerged in habitat use with respect to cover in light and dark *T. elegans* – both colour morphs were equally vulnerable in terms of visibility to predators. In the interspecific comparison, however, light *T. elegans* was more conspicuous than *T. sirtalis* and because of this, I expected light *T. elegans* would be found closer to cover. This prediction, however, was not supported – both species were found at similar distances from refuge. Despite the lack of relationships reported here, results from meta-analytical work clearly showed that animals use distances to safety when making escape decisions (Stankowich and Blumstein 2005). Why there was no relationship with proximity to refuge and dorsal colouration is unclear but perhaps distance to cover in this example forms part of a diverse assemblage of factors, in addition to crypsis, that animals use to assess risk (Warkentin and Caldwell 2009).

Distance to cover was, however, an important factor in explaining the antipredator behaviour of snakes in the field. Risk assessment is a dynamic process (Ydenberg and Dill 1986) whereby animals rely on temporal or spatial mechanisms to assess risk and modify the distance at which flight is initiated (Gulbransen et al. 2006). Species that use spatial mechanisms initiate flight based on the distances maintained from predators and/or safety (Stankowich and Blumstein 2005) whereas temporal mechanisms rely on the assessment of predator approach speed (Bonenfant and Kramer 1996). Although I did not quantify search speed *per se*, careful examination of habitats necessitated a fairly slow and constant walking effort; as such, temporal cues were presumably constant and therefore dismissed. All snakes that were found farther from cover were more likely to be moving when they were first detected as well as moving when approached. This strong relationship suggests that snakes used spatial cues when assessing the level of risk and making the decision to flee, which was independent of colour morph and species.

I originally predicted that there would be no difference in tendency to move when first detected in the field in snakes that exhibited equal crypsis (i.e. light and dark *T. elegans*). More cryptic snakes (i.e. *T. sirtalis*), on the other hand, would be less likely to move because immobility contributes to inconspicuousness from predators. Contrary to expectation, however, light *T. elegans* were more likely to move when originally detected than both dark *T. elegans* and *T. sirtalis*. What then might explain differences in tendency to move or remain still in the field? A multi-population behavioural study of zebra-tailed lizards, *Callisaurus draconoides*, and greater eared lizards, *Cophosaurus taxanus*, concluded that escape decisions were influenced by a variety of environmental cues including amount of plant cover, air temperature, distance to cover, and predator-approach qualities (Bulova 1994). Although colour was not included in this study, the results suggest that perhaps the tendency to move when detected could be related to a suite of other factors, in addition to crypsis (Stankowich and Blumstein 2005).

Risk assessment is also dependent upon individual parameters, such as performance ability (Cooper 2003b). The ability to move quickly is therefore directly linked to a snake's ability to avoid being caught. In general, snakes became faster with increasing T_bs, but differences attributable to colour morph in this study were not straightforward. For example, dark *T. elegans* were faster than light *T. elegans* across all T_bs, but male and nongravid female light *T. elegans* were significantly faster at some (notably lower) T_bs (15-16°C for males and 24°C for females). Higher speed at low T_bs may be adaptive, especially for light *T. elegans* early on in the season when snakes emerge from hibernation and are exposed to predators. Male light *T. elegans* were also significantly faster than *T. sirtalis* across a range of T_bs but this trend was reversed in nongravid females. This interaction between sex and colour and the relationship with speed at various T_bs suggests that potential tradeoffs may occur between these factors, at least in Creston where light *T. elegans* and *T. sirtalis* co-occur.

Of those motionless snakes detected in the field, I predicted that more cryptic snakes would be more likely to maintain this immobility up to the point of capture. I hypothesized that immobility would be maintained because the costs associated with unnecessary flight (i.e. attracting predator attention) would be minimized, thereby maximizing the benefits conferred by crypsis (Cooper et al. 2008a). It appeared, however, that the assessment of risk was comparable between colour morphs and between species. (Warkentin and Caldwell 2009); that is, both colour morphs and species responded equally to the threat of an approaching predator. Could there be a potential role, if any, of previous predator exposure on these observed antipredator behaviours (Martin et al. 2009)? That is, could snakes use previous exposure to predators in assessing future antipredator activities, such as the tendency to move when approached? It appears that prey can be more sensitive to predators when approached sequentially in the field (Stankowich and Blumstein 2005). Flight decisions made by the rock lizard, *Iberolacerta cyreni,* were influenced more by previous attack experience than by the degree of conspicuousness after predator detection (Martin et al. 2009). The lizard, *Acanthodactylus erythrurus,* responded to the persistent attacks of human predators by increasing the approach distances at which it fled and the amount of cover in microhabitats into which they escaped (Martin and Lopez 2003). I have shown here that snakes that exhibited some form of injury (i.e. proxy for previous predator encounters) were more likely to be found moving when first detected in the field, which was consistent between colour morphs and species. These data therefore suggest that perhaps garter snakes can interpret persistent predatory attacks as an increase in predation risk and can adjust the magnitude of their escape and antipredatory responses accordingly (Martin and Lopez 2003).

The positive relationship observed between locomotory performance and temperature has been well established in snakes (Stevenson et al. 1985, Bennett 1990) and the fact that more snakes evaded capture when T_es were high (and presumably T_bs too; see Chapter 2, Fig. 14) indicates the adaptive significance of moving quickly – that is, being able to evade predators. An alternative explanation of differences in performance of light and dark *T. elegans* and *T. sirtalis* may be more related to differences in colour pattern. Early correlational work of snake dorsal colour patterns suggested that uniformly-coloured and striped species rely on speed as a defense against predators, but that blotched or irregularly-patterned species rely initially on being motionless to prevent detection (Jackson et al. 1976). Extensive work on colour pattern variation in Northwestern Garter Snakes, *Thamnophis ordinoides*, suggested that different combinations of colour pattern and crawling behaviours interact to maximize fitness (Brodie 1992) and are demonstrated to have a genetically correlation (Brodie 1989). In particular, the degree of pattern 'stripedness' and the tendency to reverse direction while crawling were remarkably consistent among individuals and associated with high survival (Brodie 1993). Other work in lacertid lizards demonstrated that syntopic forms of *Lacerta perspicillata* exhibited different escape tactics that were correlated with divergent colour patterns (Carretero et al. 2006). Although studies incorporating both colour and pattern in animal crypsis are lacking, these examples suggest that colour pattern *per se* may have more influence on animal behaviour than background matching based on colour alone.

If crypsis and evasion fail and prey are ultimately captured, individuals can still resort to other antipredator behaviours to avoid being killed, such as aggression (Creer 2005), feigning death (Gregory et al. 2007), or increased movement to complicate prey handling. In the results presented here, a significantly higher proportion of *T. elegans* of both colour morphs exhibited some type of movement, either by twirling or thrashing their body when a predator attack was simulated. *Thamnophis sirtalis*, on the other hand, appeared to exhibit a different approach to this predicament – more snakes hung limp and motionless in the air. These two different approaches might sometimes end up in the same outcome – escape – but achieve it differently. That is, *T. sirtalis* presumably wait for the chance to be dropped, whereas *T. elegans* actively try to get away, presumably in an effort to force the predator to lose its grip and drop the snake. Once dropped, snakes can either flee or remain immobile, which presumably functions by relaxing predator alertness to prey escape thereby increasing the likelihood of escape (Gregory and Gregory 2006). There does not, however, appear to be any association with colour *per se*. It is possible, however, that my predation simulation threat was not large enough to elicit a reaction in *T. sirtalis*. In grass snakes, *Natrix natrix*, for example, individuals exhibited more elaborate death feigning responses when held by the head vs. by the tail (Gregory 2008). So, in the research presented here, perhaps an increased level of threat to snakes, particularly to the head region, may have evoked a different suite of behaviours (Langkilde et al. 2004). Even so, all snakes were subjected to the same handling regime, and theoretically the same level of predation risk, and so the resulting comparative differences are nonetheless convincing.

Research on antipredator behaviour of snakes largely involves 'human predators' (Cooper 1997a, 1998, Gregory and Gregory 2006, Gregory et al. 2007, Cooper et al. 2008a, Gregory 2008) and it is assumed that individuals respond to humans in a similar fashion as to natural predators. In many cases, this has been shown to be so (Cooper 1997a, 1998), but it is a generally untested assumption (but see Shine et al. 2000). However, occasional observation of interactions in the field between snakes and their real predators can help this assessment. For example, in Creston, BC, I observed a red-tailed hawk, *Buteo jamaicensis*, hold a gravid light *T. elegans* in its talons while perched in a nearby snag. During this entire time, the snake twirled and thrashed its body, similar to my ‘simulated’ predator attack, and the bird was constantly re-negotiating its hold. Eventually, the snake freed itself from the bird’s grip and fell through the trees, presumably to the ground below. Although I have not observed predation attempts on either *T. sirtalis* or dark *T. elegans*, it is reasonable to hypothesize that the antipredator behaviours displayed by these other two groups would be comparable to this avian attack.

What remains to be answered, however, is how garter snakes respond to other natural predators. There is widespread evidence that animals exhibit differential behaviours according to predator type (Templeton and Shriner 2003), and so we can presume the same might apply to snakes. For example, in the dragonfly, *Pachydiplax longipennis*, larvae exposed to ponds with adult dragonfly predators were more likely to exhibit movement when threatened vs. larvae from ponds with predatory fish that remained motionless (Hopper 2001). In other work on Texas horned lizards, *Phrynosoma cornutum*, lizards ran from Western Diamond Rattlesnakes, *Crotalus atrox*, and remained stationary when exposed to whipsnakes, *Masticophis* spp. (Sherbrooke 2008). It is unclear if garter snakes exhibit differential behaviours to snake predators; identifying the presence of such differential behaviours and then clarifying the different mechanisms is therefore an area deserving future research attention.

I predicted that more cryptic snakes would have fewer injuries because they would be less susceptible to predator detection and at less risk from a potential predator attack. I showed that the less cryptic light *T. elegans* had a higher probability of having an injury than *T. sirtalis* but injury patterns between the equally cryptic light and dark *T. elegans* differed by sex, so this relationship between crypsis and injury rates may not be straightforward. Although the predicted link between crypsis and injuries was not supported between colour morphs of *T. elegans*, there was a strong association between colour and injury in the interspecific comparison – the more conspicuous light *T. elegans* suffered more injuries. Predator attack rates on clay models showed that bright chameleon replicas were attacked more often than ‘dull’ models (Stuart-Fox et al. 2003). Extensive work on two colour morphs of *T. elegans*, referred to as ecotypes, in California demonstrated that light snakes do in fact have lower survivorship (Bronikowski and Arnold 1999). These light snakes, however, compensated for this with higher growth rates, earlier maturation and higher fecundity and thus, lower adult survival was offset by high reproductive rate early in life (Bronikowski and Arnold 1999, Bronikowski 2000). Although the colours of the two Californian ecotypes may superficially resemble the light and dark morphs of this study, it is unknown how crypsis compares. We should therefore be cautious in drawing parallels between colour and survivorship in this system. Regardless, the higher injury rates exhibited in light *T. elegans* suggest that a search for differential patterns of demographic tradeoffs between the colour morphs and species might be a profitable avenue for future research.

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CHAPTER 5

CONCLUSION

It has been widely demonstrated in the literature that colour is important to organisms, particularly those that live in habitats where light predominates. For example, colours influence an individual's ability to forage (Heiling et al. 2005), communicate with conspecifics (Heindl and Winkler 2003) and between species (Siddiqi et al. 2004), thermoregulate (Pereboom and Biesmeijer 2003), and avoid predators (Tordoff 1980). The adaptive significance of colour variation has long received considerable attention from the scientific community (Poulton 1890, Cott 1940) and has been intensively studied in diverse taxonomic groups such as moths (Kettlewell 1955, 1956), grasshoppers (Forsman 1997, Forsman and Appelqvist 1999), snails (Cain and Sheppard 1950, 1952), guppies (Endler 1980, 1987) and snakes (King 1987, Brodie 1992, Forsman 1995).

The research presented here provided an objective quantification of colour measurement and visual modeling approaches to investigate the relationships between dorsal colour and a suite of ecological variables in garter snakes. I evaluated potential hypotheses explaining the adaptive function of colouration both within (i.e. dark and light Western Terrestrial Garter Snakes, *Thamnophis elegans*) and between species (light *T. elegans* and Common Garter Snakes, *T. sirtalis*) that focused on three interrelated hypotheses that included thermal advantages, protection from predators, and colour-related patterns of behaviour. As demonstrated here, the relationships between colour and these various traits were not straightforward but taken together, they highlighted how thermal ecology, crypsis, and anti-predator behaviours were related to a snake's appearance in what appears to be an interconnected fashion.

Colour and temperature:

In environments where temperatures are spatially and temporally variable, it is often challenging for ectothermic animals to attain optimum body temperatures (T_bs). Because thermoregulation can be energetically expensive as well as risky if basking exposes individuals to predators, any phenotypic difference or behaviour that confers thermoregulatory advantage should be favoured. We might expect dorsal colouration, or any other factor that affects a snake's ability to achieve high T_bs, to indirectly influence individual fitness (Christian and Tracy 1981, Kingsolver and Watt 1983, Huey and Kingsolver 1989).

Quantifying colour-associated fitness benefits, such as increased fecundity and survivorship, were not the focus of the research presented here; however, patterns between colour and temperature were demonstrated. In the outdoor enclosures, gravid females of the light and dark colour morphs of *T. elegans*, exhibited comparable thermoregulatory behaviour at high temperatures, whereas dark *T. elegans* maintained elevated T_bs when available temperatures dropped. When gravid, both colour morphs were equally visible in the enclosure at lower T_bs, presumably in an effort to maximize thermal opportunities. After snakes gave birth, dark *T. elegans* had a higher probability of being visible in the enclosure and the chance of being seen increased for all nongravid snakes when T_bs were high. In the field, dark *T. elegans* and *T. sirtalis* were more likely to be moving when first detected when snakes were warm, but this trend was reversed in light *T. elegans* where cold snakes were more likely to be moving when first seen.

Body temperature data were collected from snakes in the enclosure using radiotelemetry, which provided a continuous and comprehensive profile of temperature selection for active and inactive snakes. From the enclosure experiment I concluded that, when ambient temperatures were low, dark *T. elegans* maintained significantly higher T_bs (25. 27ºC vs. 24.72ºC). Maintaining higher T_bs when ambient temperatures are cool has important implications for the survivorship of snakes because of the strong thermal dependence of performance on T_b (Stevenson et al. 1985). But, how important is a ~1°C difference in mean T_b between colour morphs? At first, this difference appears inconsequential but small differences in T_b can be biologically meaningful, especially considering that small changes in incubation temperature can affect the development of offspring traits (Shine and Harlow 1993). I would therefore argue that temperature differences and relationships observed between colour morphs of *T. elegans* are important and could contribute to individual fitness.

Future thermal studies should consider research questions on a broader scale, say across the range of *T. elegans*, to complement the smaller-scale investigations that have been completed to date (Scott and Pettus 1979, Charland 1995, O'Donnell and Arnold 2005). Not only should temperature-related studies include the influence of annual temperatures on the thermal ecology of animals (Davis et al. 2008), they should also examine how solar radiance (Clusella-Trullas et al. 2008) affects the selection of T_bs in animal groups such as garter snakes. Addressing these types of research questions are becoming highly relevant during this period of global climate change (Weaver 1993, Weaver and Hillaire-Marcel 2004).

Colour and crypsis:

Cryptic colouration is an adaptation against visually oriented predators whereby animals become indistinguishable from the background. There are many examples of animals that use colour to prevent detection (Ruxton et al. 2004) , and of the various strategies that animals use to become cryptic, background matching, or the visual similarity between an animal and its environment, is often noted as one of the principal strategies. Research in this area has progressed from correlative work describing similarities between animals and their backgrounds to novel empirical and theoretical approaches identifying tradeoffs associated with cryptic colour patterns (Lanridge 2006) and predicting the evolution of crypsis (Sherratt et al. 2007). The study of animal crypsis is a developing field and now includes more complete approaches such as the objective measurement of animal colour, background colour, and ambient light, as well as the interpretation of data in the context of what conspecifics, predators and prey can see. Although the connections between habitat use and crypsis are not completely clear yet for the example presented here, overall it appeared that snakes selected basking sites that maximized crypsis and were equally cryptic in regions where they normally occurred. Using visual modeling approaches, it became apparent that the two colour morphs of *T. elegans* and *T. sirtalis* were differentially cryptic to predator types, which may potentially reflect differences in selection pressure based on snake colouration.

The study of animal colouration has yielded interesting insights into the adaptive function of crypsis but there is, however, considerable work that needs to be completed in this area. First, detailed information is lacking about the visual sensitivities of a wide variety of terrestrial mammals and avian predators. Consequently, the capability for modeling cryptic colouration from a predator's perspective is limited to certain animal groups and we therefore are forced to make extrapolations using visual information from distantly related groups. Noninvasive molecular methods recently developed in birds, however, have provided insights into colour perception, particularly as it relates to ultraviolet and violet spectral sensitivity (Oden and Hastad 2003). Second, despite early attempts to assess the role of pattern in colour studies (Endler 1984, Godfrey et al. 1987, Sandoval 1994), objective approaches incorporating pattern were and continue to be lacking. Pattern plays a central role in the visual recognition of edges and boundaries (Osorio and Srinivasan 1991) and therefore contributes to crypsis through disruptive colouration (Schaefer and Stobbe 2006); quantifying pattern directly was omitted from my analysis and is problematic. Third, although it may be logistically complicated to study, it has been suggested that visual illusions created by moving objects (i.e. flicker fusion) may also influence an organism's ability to be cryptic (Shine and Madsen 1994, Lindell and Forsman 1996, Forsman and Appelqvist 1998). If movement is important, as dragonfly camouflage via motion (Mizutani et al. 2003) suggests, we therefore need to interpret experiments that are conducted using stationary prey with caution. And finally, it is becoming increasingly apparent that the incorporation of visual processing mechanisms is necessary to understand how viewing organisms, such as predators, not only perceive, but process and interpret incoming visual signals (Bond 2007, Stevens 2007). The consideration of visual and cognitive abilities will therefore be crucial to understand why particular colour patterns are successful in reducing detection. The application of artificial neural networks, which are data processing systems inspired by biological nerve systems, provides a novel way for researchers to incorporate predator perspective and deepen the understanding about prey colour evolution (Merilaita 2007). These examples highlight that animal colouration, and the study of crypsis in particular, remains an area that is rich in research possibilities.

Colour and behaviour:

Crypsis is a common morphological adaptation to avoid detection by predators, however, its effectiveness is sensitive to behaviour. Collectively, the results presented here suggested that crypsis was not necessarily independent of a snake's behavior. In general, light *T. elegans* had a higher probability of moving when first detected in the field, but distance to refuge and injuries also seemed to be important factors in explaining the behaviour of snakes. Following capture, a large proportion of dark and light *T. elegans* exhibited some type of movement when suspended in the air by the tail whereas more *T. sirtalis* hung motionless. And finally, larger snakes and light *T. elegans* had a higher probability of having some type of injury. This research characterized antipredator behaviours in the context of snake colouration but ultimately, this research needs to address the potential fitness implications of colour variation. Variation in colour pattern has been shown to have important implications for the evolution of life-history traits (Andren and Nilson 1981, Capula and Luiselli 1994, Ahensjo and Forsman 2003, Ellers and Boggs 2003) and demonstrating these links with behaviour would be an appropriate avenue of future research for the garter snakes studied here.

The evolution of animal colour patterns is a highly complex process, involving multiple selection pressures. Often, it is difficult to disentangle direct selection on colour (i.e. reduced predation because of crypsis) from selection on other traits (e.g. thermoregulation, activity level, or escape behavior) that may be correlated with variation in this phenotypic trait (Brodie 1992, Endler 1995, Forsman and Appelqvist 1998). For example, Ahensjo & Forsman (2006) showed how the interaction between substrate temperature and background choice varied between colour morphs of pygmy grasshoppers, *Tetrix undulata*, when predation risk was introduced. Grasshoppers selected matching microhabitats when predation risk increased but this habitat choice was mediated by the interaction with substrate temperature, which indirectly influenced grasshopper jumping performance (Ahensjo and Forsman 2006). As a result, selection may favour the evolution of correlated traits.

Jackson et al. (1976) suggested that colour pattern and behaviour were correlated in snakes and provided a comprehensive review of numerous species to support his correlative argument. Brodie (1989) further developed this research idea and demonstrated that colour and antipredator behaviour were genetically correlated in Northwestern Garter Snakes, *T. ordinoides*, which maintained colour variation in this species. Genetic correlations between colour and behaviour have also been reported in invertebrates that exhibit colour variation (Forsman and Appelqvist 1998, Ahensjo and Forsman 2003). It is therefore reasonable to expect a potential link between colour and behaviour in the garter snakes studied here not only between species but also within species where geographic variation in colour occurs.

Colour and genetics

What other mechanisms could be involved in differentiation of colour morphs within a species? In this study, I have considered possible connections between colour and temperature, crypsis, and antipredator behaviour. Ultimately, we might expect an underlying genetic mechanism contributing to the differentiation of colour morphs of *T.*

elegans. The melanocortin-1 receptor (*Mc1r*) gene has received much recent attention for its role in influencing pigmentation in a wide variety of birds (Theron et al. 2001, Mundy et al. 2004), large and small mammals (Ritland et al. 2001, Eizirik et al. 2003, Nachman et al. 2003), and lizards (Rosenblum et al. 2004). In birds, for example, the *Mc1r* gene appears to have diverse functions in waterfowl, seabirds, and passerines where it influences plumage colouration and pattern to varying degrees. For example, *Mc1r* alleles control pattern differences in lesser snow geese, *Anser C. caerulescens*, gradual melanism changes in arctic skuas, *Stercorarius parasiticus*, and abrupt melanism changes in tropical bananaquits, *Coreba flaveola* (Mundy et al. 2004). Given that this gene is involved in diverse taxonomic groups and appears to be highly conserved, it is therefore reasonable to hypothesize that genes, such as *Mc1r*, could function to control colour and pattern in snakes. The results presented here therefore provide an ecological underpinning for future genetic studies to identify potential candidate genes that may be responsible for the control of colour pattern in Western Terrestrial Garter Snakes.

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