

The Thermal Ecology of the European Grass Snake, *Natrix natrix*,  
in southeastern England

by

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A Thesis Submitted in Partial Fulfillment of the  
Requirements for the Degree of

MASTER OF SCIENCE

In the Department of Biology

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University of Victoria

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## **ABSTRACT**

What factors influence the biology and distribution of animals? The ecology of ectotherms is inevitably about how these animals interact with their thermal environment because body temperatures ( $T_b$ ; plural,  $T_{bs}$ ) are determined principally by ambient conditions. Temperature is important because it influences rates of basic biological processes (e.g., membrane transport), thus affecting physiological functions. Terrestrial ectotherms, such as reptiles, are therefore compromised when they cannot maintain  $T_{bs}$  that optimize performance. This has potential behavioural and ecological consequences that can ultimately influence fitness.

High-latitude environments are particularly challenging for ectotherms because they experience wide variations in temperature both spatially and temporally. Here I study the thermal ecology of a high-latitude natricine, the European Grass Snake, *Natrix natrix*, using field and laboratory methods, at a site near Fordwich, UK.

I collected spot  $T_b$  measurements of Grass Snakes caught in the field. Grass Snakes were active over a broad range of  $T_{bs}$  and snakes maintained relatively constant  $T_{bs}$  when environmental temperatures were high.

In order to monitor  $T_{bs}$  in a more controlled environment, I used radiotelemetry to continuously record  $T_{bs}$  of Grass Snakes in an outdoor enclosure in June-July 2002. I used physical models to estimate the environmental temperatures, or operative temperatures ( $T_e$ ; plural,  $T_{es}$ ) available to snakes. I then determined if snakes thermoregulated when given the opportunity (high  $T_{es}$ ). Snakes achieved high  $T_{bs}$  during the hottest part of the day, although these opportunities were limited ( $\approx 55\%$  of the days).

From piecewise regression, I showed that Grass Snakes initiated thermoregulation at relatively high  $T_{e,s}$  ( $T_e = 38.44$  °C,  $T_b = 27.7$  °C) and maintained fairly stable  $T_{b,s}$  (30 °C) as  $T_{e,s}$  increased. Body temperatures similar to  $T_{e,s}$  at lower  $T_{e,s}$ , however, suggested only moderate thermoregulatory behaviour at these temperatures. Contrary to expectation, nongravid females maintained significantly higher  $T_{b,s}$  than gravid females at lower  $T_{e,s}$ , however, this difference was nonsignificant at high  $T_{e,s}$ .

Maintaining optimal  $T_{b,s}$  in the field might be compromised by risks such as predation. In order to determine what  $T_{b,s}$  snakes would select in the absence of these risks, I used radiotelemetry to determine the preferred or selected temperatures ( $T_{set}$ ) of Grass Snakes in the laboratory. Body temperatures of snakes were monitored in a thermogradient over a 24-hr period during June and August 2002. Despite significant variation among individuals, all snakes maintained high  $T_{b,s}$  ( $T_{b,s} > 30$  °C). These high  $T_{set}$  values exceed those reported in the literature for other natricines.

I then asked whether  $T_{b,s}$  maintained by snakes in the field and chosen in the laboratory coincided with  $T_{b,s}$  where performance is optimized. I tested performance of 3 functions (crawling, swimming & tongue-flick rate) over a range of  $T_{b,s}$  in the laboratory. Mean and maximum speeds of crawling and swimming were highly temperature-dependent and maximum speeds occurred over a broad range of high  $T_{b,s}$  that included temperatures maintained in the field and in the laboratory. Tongue-flick rates, however, appeared to be relatively independent of temperature but I cannot rule out experimental design flaws in this case.

Overall, the data I have collected suggest that the thermal ecology of Grass Snakes in Fordwich, UK is similar to that of other temperate-zone natricines.



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## ACKNOWLEDGEMENTS

I would like to thank my supervisor, Pat Gregory, for showing me how important it is to always keep your question in mind when doing research. He has helped me develop both my critical thinking and writing skills and I am grateful. I am indebted to my husband, Doug Schneider, who was instrumental in catching animals, completing laboratory experiments, being extremely patient, and for being an overall awesome guy. I am grateful to Dr. R.A. Griffiths of the University of Kent at Canterbury, Canterbury, Kent, UK for his logistical support of my project. Thank you to Pat Kerfoot of the Biology Electronics Laboratory for his technical assistance and to Colin May of Faversham, Kent, UK for generously providing all weather data. Eleanore Floyd of the Biology Department provided invaluable support throughout my graduate career.

This research was financially supported by an N.S.E.R.C. PGS A Scholarship held by L. A. Isaac and an N.S.E.R.C. Research Grant held by P. T. Gregory. Funding was also provided by the University of Victoria.

## GENERAL INTRODUCTION

Temperature is a major physical factor influencing the biology and distribution of animals (Root, 1988; Humphries *et al.*, 2002). Because of its influence on the rates of biological processes (e.g., membrane function: Gracey, *et al.*, 1996), temperature affects physiological functions and, by extension, when and where animals can be active (Chappell & Bartholomew, 1981; Grant & Dunham, 1988). Animals are compromised when they cannot maintain body temperatures ( $T_b$ ; plural,  $T_{bs}$ ) that optimize performance. This has potential behavioural and ecological consequences that can ultimately influence fitness (Christian & Tracy, 1981; Beaupre, 1995). High latitudes and high altitudes are therefore challenging environments because they experience wide variations in temperature, both temporally and spatially (Huey & Kingsolver, 1989; Blouin-Demers & Weatherhead, 2001). Such environments are particularly challenging for ectotherms, or cold-blooded animals, because they have a relatively limited physiological capacity for generating significant heat metabolically (Hutchison *et al.*, 1985). Rather, ectotherms rely on external sources of heat to maintain  $T_{bs}$ .

Terrestrial ectotherms, such as reptiles, potentially can meet the challenges of a in various ways that are not mutually exclusive. They can: 1. avoid lethally extreme surface temperatures by hibernating or aestivating (Gregory, 1984; Grant & Dunham, 1988), 2. tolerate a wide range of  $T_{bs}$  allowing activity over a wider range of  $T_e$ s (Kingsbury, 1994; Dorcas, 1998), and 3. thermoregulate, when possible, to maintain optimum  $T_{bs}$ . In reptiles, behavioural thermoregulation, such as microhabitat selection and postural adjustments, is typically more important than physiological mechanisms in controlling  $T_{bs}$  (Stevenson, 1985). Because the main thermal challenges facing reptiles are in temperate zones rather than the tropics (Shine &

Madsen, 1996), reptiles with northerly distributions provide an opportunity to investigate issues in thermal biology where potential 'costs' are perhaps more extreme. An important question is the extent to which temperate-zone reptiles are able to thermoregulate.

In order to evaluate thermoregulatory patterns, three different temperature measurements should be collected: 1. body temperature,  $T_b$ , describes the temperature of an animal in a given environment, 2. operative temperatures ( $T_e$ ; plural,  $T_{eS}$ ) describe the environmental temperatures that are available for an animal to exploit, and finally, 3. preferred temperature,  $T_{set}$ , describes the  $T_b$  an animal would select when given a wide range of temperature options.

Ultimately, thermoregulation should have fitness consequences. One explicitly fitness-oriented model of thermoregulation is that of Huey and Slatkin (1976), who argue that the extent to which reptiles thermoregulate reflects the relative 'benefits' and 'costs' associated with such behaviour as well as the constraints imposed by the environment (Grant & Dunham, 1988; Christian & Bedford, 1995). Benefits include physiological or ecological advantages that arise from thermoregulating, such as accelerated rates of development and increased copulatory frequency that ultimately influence the fitness of an individual. Thermoregulatory costs, on the other hand, include disadvantages that an individual incurs, such as increased predation risk and increased energy expenditure that have potential negative fitness consequences. Huey and Slatkin (1976) suggest that reptiles should stop thermoregulating, and thermoconform to the thermal environment, when these costs outweigh the benefits. It is generally assumed that thermoregulatory costs are relatively high in the temperate zone, largely because environmental temperatures are highly variable and thermoregulatory opportunities may be somewhat limited. Recent

work (Blouin-Demers & Weatherhead, 2001), however, suggests that reptiles in thermally challenging temperate-zone environments can be moderately effective thermoregulators overall. More effective thermoregulators maintain  $T_b$ s in the field that are closer, on average, to preferred  $T_b$ s that are measured in the laboratory.

Another factor influencing thermoregulatory behaviour is the physiological state of an individual (Charland & Gregory, 1990; Daut & Andrews, 1993; Burns *et al.*, 1996). Much attention has been focused on reproductive state because developmental rate, viability of offspring and phenotype of offspring are all markedly affected by temperature (van Damme *et al.*, 1992; Rock & Cree, 2003). Thus, gravid females of viviparous snakes usually are found to thermoregulate more precisely around a given  $T_b$  than do non-gravid females in the same environmental conditions (Beuchat, 1986; Charland, 1995; Brown & Weatherhead, 2000). Although females of oviparous species retain eggs for a shorter period of time than do viviparous species, they also might be expected to thermoregulate in a similar fashion while gravid. Few studies have been done of oviparous species, but recent work (Blouin-Demers & Weatherhead, 2001) supports this prediction.

Although most species of snakes are oviparous ( $\approx 80\%$ ), viviparous species predominate at high latitudes and altitudes. This is presumably because viviparous females can maintain the best possible thermal conditions for their developing offspring by retaining them throughout the entire pregnancy ('cold-climate' hypothesis; Shine, 1985, 1987a, b). Oviparous species, by contrast, can use thermoregulation to optimize development of offspring only while eggs are *in utero*; following oviposition, further development depends on ambient temperatures in the nest site (Vinegar, 1974; Gutzke & Packard, 1987), which may be quite variable at high latitudes. Thus, oviparous species are rare or absent at high latitudes.

The European Grass Snake, *Natrix natrix*, is one of the most common and widespread snakes in Europe, ranging from northern Africa to near the Arctic Circle in Scandinavia. Thus, it occurs in a wide range of thermal environments making it ideal for comparative geographic studies. It also is ecologically similar and phylogenetically related to North American snakes of the genus *Thamnophis* (garter snakes), which have been widely used in studies of thermal ecology (Hawley & Aleksasuk, 1975; Gregory, 1977; Gibson & Falls, 1979; Lysenko & Gillis, 1980; Gibson *et al.*, 1989), including tests of the cold-climate hypothesis (Charland, 1995). By contrast, the thermal ecology of *Natrix natrix* is relatively unstudied, except for occasional field observations (Mertens, 1994; Gentilli & Zuffi, 1995). *Natrix* also differs from *Thamnophis* in reproductive mode (*Natrix*: egg-laying; *Thamnophis*: live-bearing), and thus offers an opportunity to test a logical corollary of the cold-climate hypothesis: that high-latitude oviparous snakes should thermoregulate in a similar fashion to viviparous snakes while gravid.

In this thesis, I focus on several aspects of the thermal ecology of *Natrix natrix* in southern England. The specific questions I ask are:

1. How does performance vary with temperature?
2. What  $T_b$ s do snakes select when given a wide range of temperatures from which to choose?
3. How often can snakes reach their presumed optimal  $T_b$ s?
4. What thermoregulatory patterns do snakes display in a semi-natural environment?
5. Do gravid females thermoregulate differently from nongravid females?

## LITERATURE CITED

- Beuchat, C. A. 1986. Reproductive influences on the thermoregulatory behaviour of a live-bearing lizard. *Copeia* **1986**(4): 971-979.
- Beaupre, S. J. 1995. Effects of geographically variable thermal environment on bioenergetics of Mottled Rock Rattlesnakes. *Ecology* **76**(5): 1655-1665.
- Blouin-Demers, G. & Weatherhead, P. J. 2001. Thermal ecology of black rat snakes (*Elaphe obsoleta*) in a thermally challenging environment. *Ecology* **82**(11): 3025-3043.
- Brown, G. P. & Weatherhead, P. J. 2000. Thermal ecology and sexual size dimorphism in Northern Water Snakes, *Nerodia sipedon*. *Ecol. Mono.* **70**(2): 311-330.
- Burns, G., Ramos, A. & Muchlinski, A. 1996. Fever response in North American snakes. *J. Herp.* **30** (2): 133-139.
- Chappell, M. A. & Bartholomew, G. A. 1981. Activity and thermoregulation of the Antelope Ground Squirrel, *Ammospermophilus leucurus*, in winter and summer. *Physiol. Zool.* **54**(2): 215-223.
- Charland, M. B. 1995. Thermal consequences of reptilian viviparity: Thermoregulation in gravid and nongravid Garter Snakes (*Thamnophis*). *J. Herp.* **29**(3) 383-390.
- Charland, M. B. & Gregory, P. T. 1990. The influence of female reproductive status on thermoregulation in viviparous snake, *Crotalus viridis*. *Copeia* **1990**: 1089-1098.
- Christian, K. A. & Tracy, C. R. 1981. The effect of the thermal environment on the ability of hatchling Galapagos Land Iguanas to avoid predation during dispersal. *Oecologia (Berl)* **49**: 218-223.
- Christian, K. A. & Bedford, G. S. 1995. Seasonal changes in thermoregulation by the Frillneck Lizard, *Chlamydosaurus kingii*, in tropical Australia. *Ecology* **76** (1): 124-132.
- Daut, E. F. & Andrews, R. M. 1993. The effect of pregnancy of thermoregulatory behaviour of the viviparous lizard *Chalcides ocellatus*. *J. Herp.* **27**(1): 6-13.
- Dorcas, M. E. 1998. Daily temperature variation in free-ranging rubber boas. *Herpetologica* **54**(1): 88-103.
- Gentilli, A. & Zuffi, M. A. L. 1995. Thermal ecology of a Grass Snake (*Natrix natrix*) population in Northwestern Italy. *Amphibia-Reptilia* **16**: 401-404.

- Gibson, A. R. & Falls, J. B. 1979. Thermal biology of the common garter snake *Thamnophis sirtalis*. I. Temporal variation, environmental effects and sex differences. *Oecologia* **43**: 79-97.
- Gibson, A. R., Smucny, D. A., & Kollar, J. 1989. The effects of feeding and ecdysis on temperature selection by young Garter Snakes in a simple thermal mosaic. *Can. J. Zool.* **67**: 19-23.
- Gracey, A. Y., Logue, J., Tiku, P. E. & Cossins, A. R. 1996. Adaptation of biological membranes to temperature: biophysical perspectives and molecular mechanisms. In *Animals and Temperature: Phenotypic and Evolutionary Adaptation*. (Johnston, A. & Bennett, A. F. eds.). Cambridge University Press, New York. pp: 1-23.
- Grant, B. W. & Dunham, A. E. 1988. Thermally imposed time constraints on the activity of the desert lizard *Sceloporous merriami*. *Ecology* **69**(1): 167-176.
- Gregory, P. T. 1977. Life-history parameters of the Red-Sided Garter Snake (*Thamnophis sirtalis parietalis*) in an extreme environment, the Interlake region of Manitoba. *Natl. Mus. Can. Publ. Zool.* **13**: 1-44.
- Gregory, P. T. 1984. Communal denning in snakes. In *Vertebrate Ecology and Systematics: A Tribute to Henry S. Fitch*. (Seigel, R. A., Hunt, L. E., Knight, J. L., Malaret L., & Zuschlag, N. L. eds). Univ. Kans. Mus. Nat. Hist. Spec. Pub. 10: 57-75.
- Gutzke, W. H. N. & Packard, G. C. 1987. Influence of the hydric and thermal environments on eggs and hatchlings of Bull Snakes, *Pituophis melanoleucus*. *Physiol. Zool.* **60**(1): 9-17.
- Hawley, A. & Aleksyuk, M. 1975. Thermal regulation of spring mating behaviour in the Red-Sided Garter Snake (*Thamnophis sirtalis parietalis*). *Can. J. Zool.* **52**: 768-776.
- Huey, R. B. & Kingsolver, J. G. 1989. Evolution of thermal sensitivity of ectotherm performance. *TREE* **4**(5): 131-135.
- Huey, R. B. & Slatkin, M. 1976. Cost and benefits of lizard thermoregulation. *Quart. Rev. Biol.* **51**(3): 363-384.
- Humphries, M. M., Thomas, D. W., & Speakman, J. R. 2002. Climate-mediated energetic constraints on the distribution of hibernating mammals. *Nature* **418**: 313-316.
- Hutchison, V. H., Dowling, H. G., & Vinegar, A. 1966. Thermoregulation in a brooding female python, *Python molurus bivittatus*. *Science* **151**: 694-696.
- Kingsbury, B. A. 1994. Thermal constraints and eurythermy in the lizard *Elgaria multicarinata*. *Herpetologica* **50**(3): 266-273.

- Lysenko, S. & Gillis, J. E. 1980. The effect of ingestive status on the thermoregulatory behaviour of *Thamnophis sirtalis sirtalis* and *Thamnophis sirtalis parietalis*. *J. Herp.* **14**(2): 155-159.
- Mertens, D. 1994. Some aspects of thermoregulation and activity in free-ranging Grass Snakes (*Natrix natrix* L.). *Amphibia-Reptilia* **15**: 322-326.
- Rock, J. & Cree, A. 2003. Intraspecific variation in the effect of temperature on pregnancy in the viviparous gecko, *Hoplodactylus maculatus*. *Herpetologica*. **59**(1): 8-22.
- Root, T. 1988. Environmental factors associated with avian distributional boundaries. *J. Biogeogr.* **15**: 489-505.
- Shine, R. 1985. The evolution of viviparity in reptiles: An ecological analysis. In *Biology of the Reptilia*, vol. 15. (Gans, C. & Billet, F. eds.). John Wiley and Sons, New York. pp. 605-694.
- \_\_\_\_\_. 1987a. Reproductive mode may determine geographic distributions in Australian venomous snakes (*Pseudechis*, Elapidae). *Oecologia (Berl)* **71**: 608-612.
- \_\_\_\_\_. 1987b. The evolution of viviparity: ecological correlates of reproductive mode within a genus of Australian snakes (*Pseudechis*, Elapidae). *Copeia* **1987**: 551-563.
- Shine, R. & Madsen, T. E. 1996. Is thermoregulation unimportant for most reptiles? An example using water pythons (*Liasis fuscus*) in tropical Australia. *Physiol. Zool.* **69**(2): 252-269.
- Stevenson, R. D. 1985. The relative importance of behavioural and physiological adjustments controlling body temperature in terrestrial ectotherms. *Am. Nat.* **126**(3): 362-386.
- van Damme, R., Bauwens, D., Brana, F., & Verheyen, R.F. 1992. Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. *Herpetologica* **48**(2): 220-228.
- Vinegar, A. 1974. Evolutionary implications of temperature induced anomalies of development in snake embryos. *Herpetologica* **30**: 72-74.

## **CHAPTER 1: Natural History and Thermal Ecology of the European Grass Snake, *Natrix natrix*, in southeastern England**

### **INTRODUCTION**

The design and interpretation of experimental results in biology requires an appropriate context. That is, are the conditions imposed in the experiment within or outside the range of those that the organism might be expected to encounter in the wild? Natural history, the study of organisms in their natural settings, provides that context (Greene, 1986). Natural history also plays a key role in the development of theory and in testing hypotheses derived from theory (Greene, 1986). The questions addressed by natural historians pervade most disciplines of contemporary biology - ecology, evolutionary biology and population genetics (Bartholomew, 1986); natural history thus is the vehicle by which these disparate areas can be integrated.

The thermal ecology of ectotherms, such as snakes, is inevitably about how animals interact with their physical environment because body temperatures ( $T_b$ ; plural,  $T_b$ s) are determined principally by ambient conditions rather than by metabolic generation of heat. Determination of the conditions under which such animals are active in the field thus provides essential background for designing and interpreting experiments to test ideas about temperature choice and thermoregulation.

In this thesis, I address various questions about the thermal ecology of the Grass Snake, *Natrix natrix*, a widespread and abundant species in Europe. Wide-ranging species are especially interesting because they are likely to be "broad-niched" (Brown, 1995), tolerating, for example, a wide range of thermal conditions. Despite its abundance, however, no comprehensive study has been done of the thermal ecology of the Grass Snake. Such study should complement and test the generality of work done on other high-latitude taxa of snakes, especially the related and

ecologically similar, and well-studied Garter Snakes (*Thamnophis*) of North America (Hawley & Aleksyuk, 1975; Gregory, 1977; Gibson & Falls, 1979; Lysenko & Gillis, 1980; Gibson *et al.*, 1989).

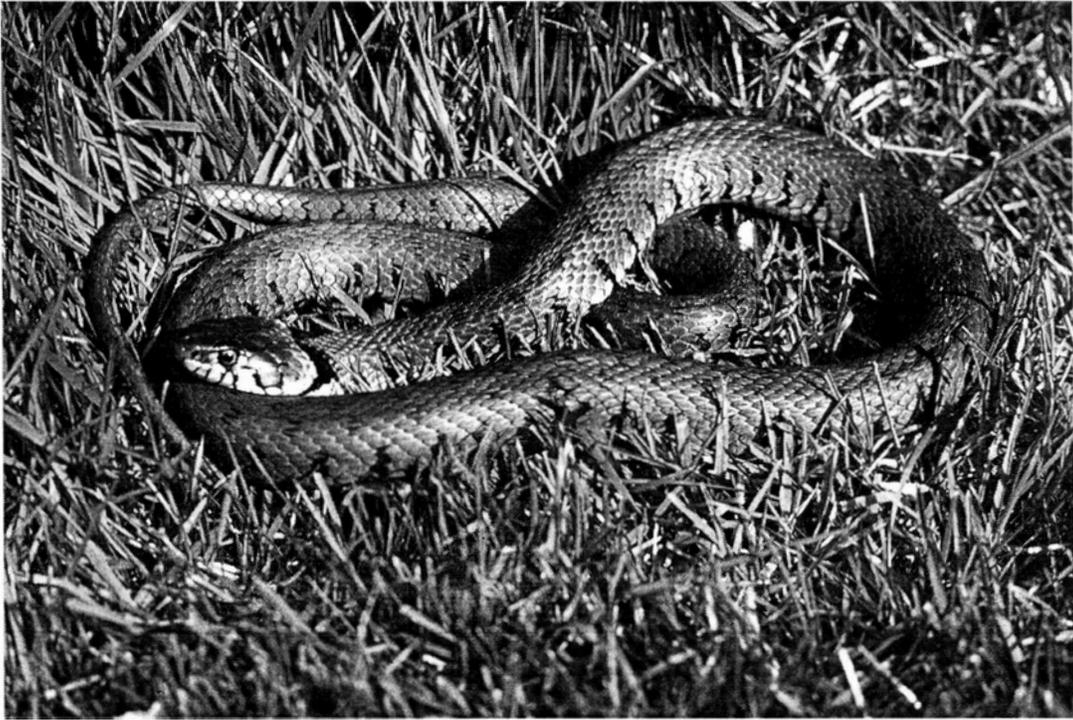
Here I describe the general natural history of the Grass Snake, with a particular focus on characterizing the body temperatures of free-ranging animals at a site in southeastern England.

## **STUDY SPECIES**

The Grass Snake is widely distributed across much of Europe. It ranges from Lake Baikal (Russia) in the east to western Portugal and from northern Africa to southern Scandinavia, including England and various Mediterranean islands (Beebe & Griffiths, 2000). Grass Snakes are slate-green in colour with a distinctive yellow and black band around the neck and black marks extending down the dorsal side (Fig. 1). Adult Grass Snakes range in body size from 500 to 800 mm. As in other natricine snakes (Garter Snakes, Gregory, 1977; Water Snakes, Weatherhead *et al.*, 1995), females are significantly larger than males (Luiselli *et al.*, 1997). Fecundity is strongly related to female body size, with larger females producing larger clutches (between 4-24 eggs; Madsen, 1987; Luiselli *et al.*, 1997).

Grass Snakes are generalist predators preying upon anurans, small mammals, birds and fish (Reading & Davies, 1996; Luiselli *et al.*, 1997; Gregory & Isaac, in press). There is relatively little geographic variation in the major prey categories of reported diets; amphibians, particularly anurans (i.e., frogs and toads), are typically the most frequent prey type (Brown, 1991; Reading & Davies, 1996; Luiselli *et al.*, 1997).

Seasonal activity begins with emergence from hibernation typically by males in early spring (Luiselli *et al.*, 1997). Mating takes place in early to late spring and



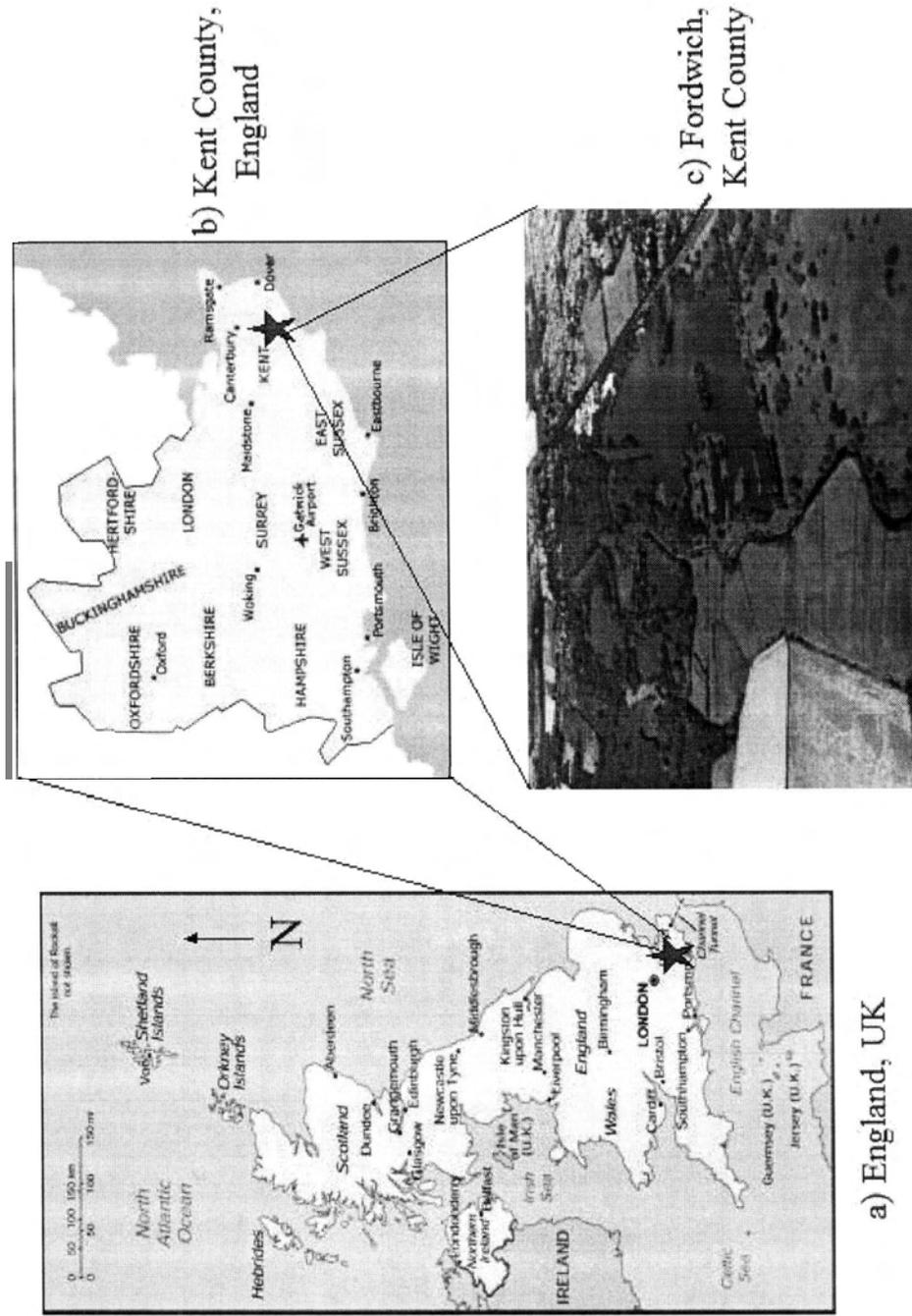
**Fig. 1.** The Grass Snake, *Natrix natrix*. (Photo: R. A. Griffiths)

male movements during this period can be extensive (mean distance travelled:  $54.8 \pm 16.8$  m SD per day; Madsen, 1984). Female movements are typically most extensive immediately prior to and after oviposition (mean distance travelled:  $114.4 \pm 74.5$  m SD per day), which presumably occurs in mid-summer (Madsen, 1984). Hatching occurs in late summer to early fall and hibernation is thought to begin by late fall (Luiselli & Shine, 1997).

Grass Snakes exhibit an unusual behaviour called death-feigning. This behaviour has been observed in other species such as the Western Hognose Snake (Burghardt & Greene, 1988) and the Western Whip Snake (Rugiero, 1999). Death-feigning may include all or some of the following components: limp body, rolled eyes, everted cloaca, extended tongue, and/or spontaneous bleeding from the mouth. One possible hypothesis for this behaviour is that it is an anti-predator strategy but it has not been studied in any detail.

## STUDY SITE

I studied a population of Grass Snakes in southern England, near Fordwich, 3.25 km NE of Canterbury, Kent, UK ( $51^{\circ}N$   $1^{\circ}E$ , 5 m elevation) (Fig. 2). Although there is considerable variability in climatic conditions between months, the temperate climate of this region is characterized by cool, wet winters and warm, dry summers. My study area encompassed habitats on either side of the River Stour and included predominantly second-growth deciduous woods and open fields with walking paths and fishing spots. The principal vegetation was Stinging Nettles (*Urtica dioica*) and Common Reed (*Phragmites australis*) as well as various species of brambles (*Rubus* sp.). Large portions of the site were once used for gravel extraction, these quarries have since filled with water to form small lakes. This network of ponds provided ideal habitat for Grass Snakes and their prey (Fig. 3 a, b).



**Fig. 2.** Map of England and Kent County and aerial view of the study site in Fordwich, UK. (Maps: <http://www.map.free.gk.com/england/england.php>, Aerial photograph: <http://www.naturegrid.org.uk/gtstour/photos-ar.html>)



a)



b)

**Fig. 3.** Typical microhabitats used by Grass Snakes. a) Branch piles and blackberry bushes and b) stinging nettles make ideal retreat sites. (Photos: P. T. Gregory)

## METHODS

I caught Grass Snakes opportunistically by hand and by flipping cover objects. Immediately following each capture, I measured the snake's cloacal temperature (body temperature,  $T_b$ ) using a mercury thermometer. I also measured operative temperatures ( $T_e$ ; plural,  $T_{es}$ ) at the snake's capture site. Operative temperature provides an estimate of the potential  $T_b$ s a snake could achieve if it thermoconformed to its thermal environment. Thus, a thermoconforming snake will have  $T_b=T_e$ , whereas a thermoregulating snake will have  $T_b \neq T_e$ . Physical models, such as copper pipe models with thermocouples inserted inside, are usually used to measure  $T_e$  (Peterson *et al.*, 1993). To measure  $T_e$  of field-captured snakes, I placed the mercury thermometer, inserted inside its metal casing, in the same location the snake was caught and I allowed it to equilibrate for at least one minute before reading the temperature.

Next, I measured a set of standard variables. I determined sex by examining the shape of the tail (female tails taper immediately following the vent) or by using a probe (probe extends down tail if a male). I measured snout-vent-length (SVL) and tail length to the nearest 1 mm by stretching the snake along a metre stick. Head diameter was determined by placing the snake's head into the different sized holes in an inking template. Body mass was measured using a hand-held Pesola spring scale. I palpated female snakes to determine the presence or absence of ovarian follicles or eggs and to estimate their numbers if present. Presence of stomach contents was obtained in a similar fashion, by gentle palpation. I scored whether injuries were present or absent and whether snakes exhibited any signs of death-feigning behaviour. In order to compare injury rates and death-feigning behaviour between different sizes,

I divided the sample into two size groups (small and large snakes) by the median SVL (699 mm).

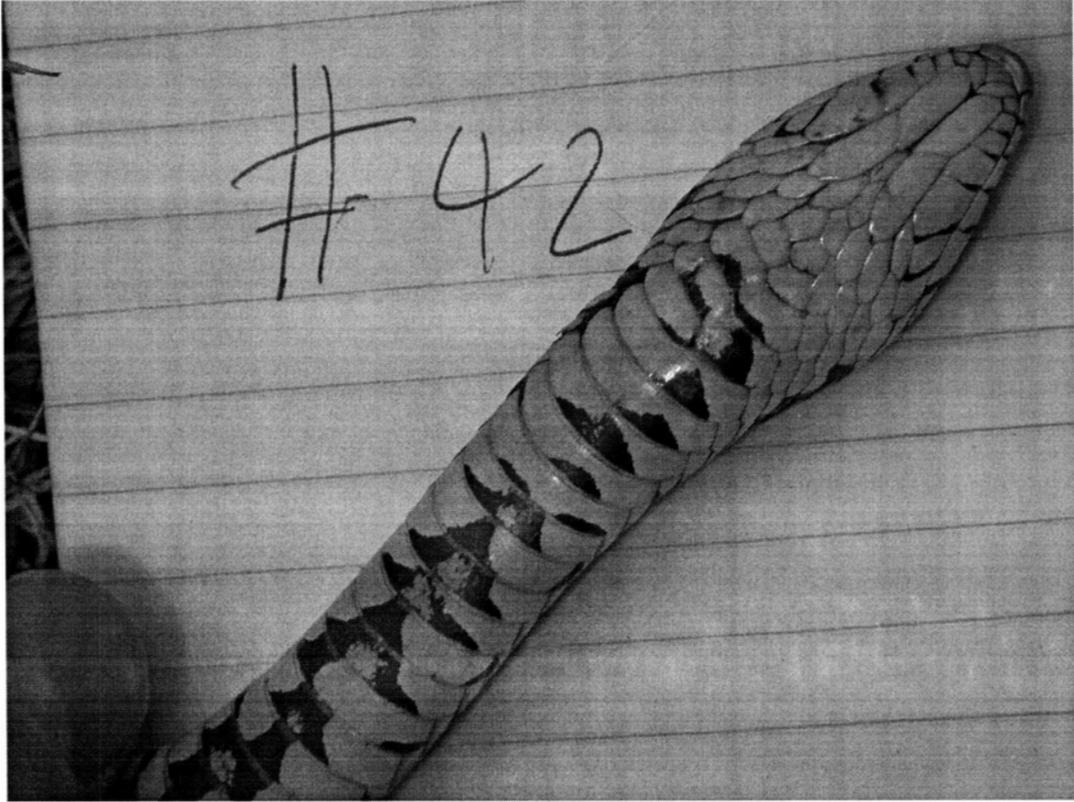
Finally, I took a photograph of the unique patterns on the ventral side of each snake to be used for identification of individual animals (Fig. 4).

Because snakes that are warm also are faster, they might elude capture more easily. If so, spot-temperature data taken in the field will be biased towards cooler individuals. This idea can be tested, at least partially, by comparing  $T_e$ s of snakes that were caught vs. those that were seen but not captured. I collected  $T_e$  data for snakes that were not captured by placing the mercury thermometer, inserted inside its metal casing, in the same location each snake was originally seen. I again allowed the thermometer to equilibrate for at least one minute before reading the temperature.

## RESULTS

In 2002, the mean annual temperature ( $10.5 \pm 3.78$  °C SD) and the mean summer temperature (May to August;  $14.6 \pm 2.0$  °C SD) did not significantly differ from yearly and summer averages respectively, from 1999, 2000 & 2001 (annual:  $F_{(3, 47)} = 0.14$ ,  $P = 0.94$ , summer:  $F_{(3, 15)} = 0.17$ ,  $P = 0.92$ ). Similar patterns were also observed for rainfall (annual:  $67.7 \pm 35.2$  mm SD;  $F_{(3, 47)} = 0.76$ ,  $P = 0.53$ , summer:  $60.9 \pm 29.5$  mm SD;  $F_{(3, 15)} = 0.04$ ,  $P = 0.99$ ). All weather data were collected in Faversham, Kent County, approximately 50 km from my study site (Colin May: <http://www.cj4386.demon.co.uk/weather/weather.htm>). Thus, the weather to which snakes were exposed during my study was broadly typical of the study site.

From May to August 2002, I caught 63 Grass Snakes (28 males, 35 females) in a variety of different microhabitats such as open patches of vegetation, fallen tree trunks, and piles of dead blackberry bushes. Grass Snakes are semi-aquatic and I saw them retreat to water when threatened, but I did not observe them in open water.



**Fig. 4.** Unique colouration pattern of the ventral side of a Grass Snake.  
(Photo: P. T. Gregory)

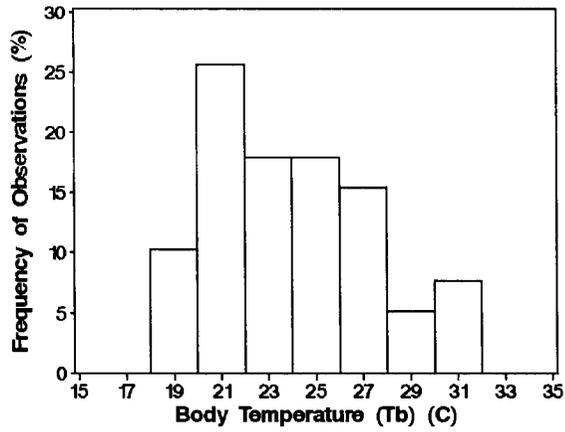
I caught snakes with a wide range (18 °C to 31.5 °C) of  $T_{bs}$  (mean  $T_b$ :  $23.5 \pm 3.5$  °C SD), but I rarely encountered snakes when conditions were extremely hot ( $T_{es} > 35$  °C; Fig. 5).  $T_{bs}$  and  $T_{es}$  were significantly correlated ( $r = 0.63$ ,  $P < 0.0001$ ,  $n = 43$ ). However, the relationship between  $T_b$  and  $T_e$  was best described by a polynomial (i.e., quadratic) regression, which was significant ( $r^2 = 0.47$ ,  $F_{(2,42)} = 17.71$ ,  $P < 0.0001$ ; Fig. 6).  $T_{bs}$  matched  $T_{es}$  closely at low  $T_{es}$ , however,  $T_{bs}$  were independent of  $T_{es}$  at higher  $T_{es}$ , suggesting thermoregulation.

Contrary to my expectation, the frequency of low and high  $T_{es}$  (divided at the median  $T_e$ , 24.0 °C) was similar for snakes that I caught and snakes that were seen but not caught (Chi-Square:  $\chi^2 = 0.2519$ ,  $df = 1$ ,  $P = 0.6158$ ). There was, therefore, no evidence that captures were biased towards snakes active under cooler conditions.

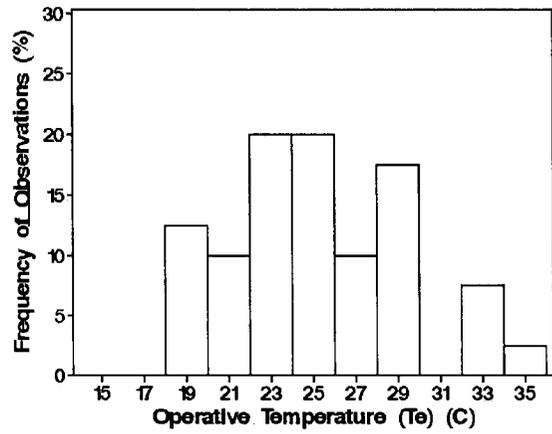
Females were significantly longer than males (ANOVA:  $F_{(1,62)} = 9.89$ ,  $P = 0.003$ ; Table 1). When adjusted for SVL, tail length also significantly differed between the sexes (ANCOVA:  $F_{(1,58)} = 7.84$ ,  $P = 0.007$ ; slopes equal). Males had relatively longer tails than females. Females had significantly larger heads than males (ANCOVA:  $F_{(1,59)} = 229.70$ ,  $P < 0.0001$ ; slopes equal) and were consistently heavier than males relative to their body size (ANCOVA: ( $F_{(1,57)} = 54.54$ ,  $P < 0.0001$ ; slopes equal).

Approximately half of the Grass Snakes (30 of 62 animals) captured had food in their stomachs. The most frequent prey were amphibians, all anurans (Table 2). The introduced Marsh Frog, *Rana ridibunda*, was the prey item most frequently eaten.

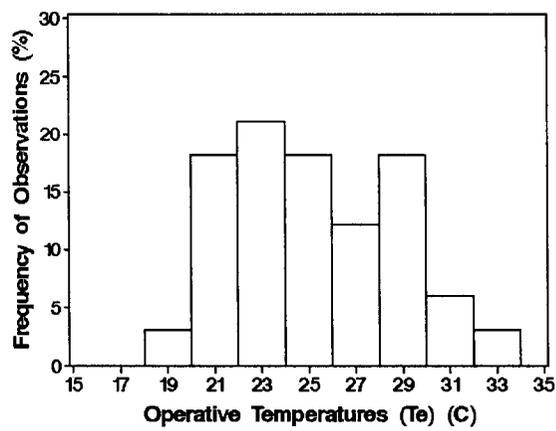
Approximately 52 % of the animals captured (33 of 63 animals) had evidence of injuries of some type, including healed bone breaks, scars in various regions of the body as well as recent injuries (i.e., open wounds). I did not witness any direct predation attempts but presumably they are avian (Madsen, 1987). There was no



a)

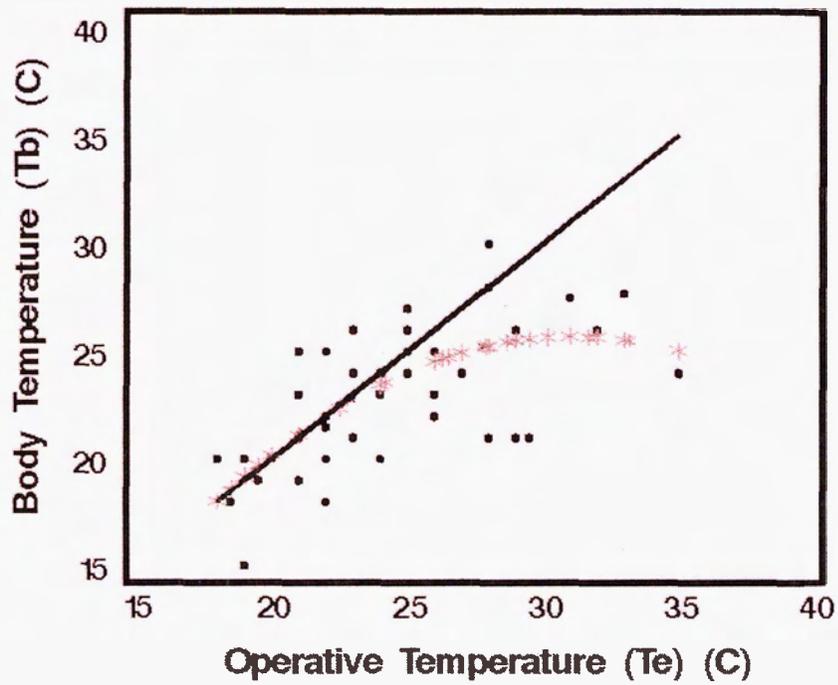


b)



c)

**Fig. 5.** a) Body temperatures ( $T_{b}$ s) of snakes caught in Fordwich, UK, and b) operative temperatures ( $T_{e}$ s) at point of capture. c) Operative temperatures ( $T_{e}$ s) measured when snakes escaped capture.



**Fig. 6.** Scatterplot of body temperatures ( $T_{bs}$ ) versus operative temperatures ( $T_{es}$ ) at Fordwich, UK. Each dot represents a single field measurement. The fitted quadratic line is indicated by red '\*'s and the reference line (slope=1) is indicated by the straight black line.

**Table 1.** Morphometric data for Grass Snakes at Fordwich, UK. Females were larger than males, on average. Females with stump tails were excluded from the tail length analysis. Only snakes weighed with no food item were included in the weight summary.

<b>Variable</b>	<b>Sex</b>	<b>n</b>	<b>Mean</b>	<b>SD</b>	<b>Min</b>	<b>Max</b>
<b>SVL</b>	Males	28	596.2	134.5	280.0	740.0
	Females	35	704.8	139.1	232.0	958.0
<b>Tail Length</b>	Males	28	143.8	32.7	64.0	208.0
	Females	34	155.0	29.3	63.0	184.0
<b>Weight</b>	Males	21	80.2	41.6	8.5	142.0
	Females	27	145.3	62.7	5.3	274.0
<b>Head Diameter</b>	Males	28	11.7	2.8	6.0	14.0
	Females	35	15.2	3.1	5.5	20.0

**Table 2.** Summary of food items found in stomachs of Grass Snakes at Fordwich, UK. One animal was severely injured and therefore not included in the analysis.

<b>Prey Type</b>	<b>Number of Snakes Containing Prey Type</b>
Amphibians	
Toads	5
Frogs	13
Small Mammals	7
Birds	1
Unknown item	2
No food	34
<b>TOTAL SNAKES</b>	<b>62</b>

significant difference in injury rate between the sexes (Chi-Square:  $\chi^2=0.36$ ,  $df=1$ ,  $P=0.55$ ). There was, however, a significant difference in injury rates between size groups. Larger snakes (22 of 33 animals) had significantly more injuries than smaller snakes (Chi-Square:  $\chi^2=8.45$ ,  $df=1$ ,  $P=0.004$ ).

Only 25 of 62 animals (40 %) death-feigned upon capture. There was no significant difference in the occurrence of death-feigning either between the sexes (Chi-Square:  $\chi^2=1.43$ ,  $df=1$ ,  $P=0.23$ ) or between different sized snakes (Chi-Square:  $\chi^2=2.57$ ,  $df=1$ ,  $P=0.11$ ).

## DISCUSSION

Overall, the data I collected suggest that Grass Snakes at Fordwich had a similar natural history to that described for populations elsewhere. Common features of this species' natural history include a diet principally of anurans (Reading & Davies, 1996), pronounced sexual size dimorphism (Madsen & Shine, 1997), and a broad range of activity temperatures (Mertens, 1994). Injury rates have not previously been reported in this species, but the pattern I observed is similar to that reported for other species of natricines, in which larger, presumably older, animals have a higher frequency of injuries (Fitch, 2003). Death-feigning was observed in a high proportion of the animals captured, but was not related to either sex or size of animal.

Interpreting body temperature variation of field-active animals in the context of an animal's natural history, including available environmental temperatures, can provide insights into thermal strategies used by reptiles (Peterson *et al.*, 1993). One important generalization concerning body temperature ( $T_b$ ) variation is that when possible, many species maintain  $T_b$ s within a relatively narrow range (stenothermy), using behavioural mechanisms (Brattstrom, 1965; Avery 1982; Stevenson, 1985).

Although the necessity of thermoregulation for most reptiles has been debated in the literature (Shine & Madsen, 1996; Akani *et al.*, 2002; Luiselli & Akani, 2002), the benefits of such behaviour include enhanced locomotory abilities (Stevenson, *et al.*, 1985), increased rates of digestion (Skocylas, 1970; Lillywhite, 1987), and accelerated growth and developmental rates (van Damme *et al.*, 1992; Autumn & de Nardo, 1995).

Despite the benefits of stenothermy, some species have highly variable  $T_{bs}$  during periods of activity (eurythermy), particularly species living in thermally variable environments (e.g., high latitudes or high altitudes; Garter Snakes, Peterson, 1987; Rubber Boas, Dorcas & Peterson, 1998; Water Snakes, Brown & Weatherhead, 2000, Rat Snakes: Blouin-Demers & Weatherhead, 2001). In this study, I have shown that Grass Snakes are active over a broad range of  $T_{bs}$  (18 °C to 31.5 °C), consistent with previous work on populations in Germany and Italy (Mertens, 1994; Gentilli & Zuffi, 1995). Despite variation in  $T_{bs}$ , it is evident that Grass Snakes are not simply thermally passive;  $T_{bs}$  plateau at high  $T_{es}$  as snakes thermoregulate and presumably seek refuge from lethally high ambient temperatures. Thus, it is likely that  $T_{bs}$  of snakes I caught did not differ significantly from those that I did not catch. This conclusion is supported by the lack of significant differences between the  $T_{es}$  of each group. Presumably, my failure to catch some snakes depended on other details of the encounter.

What are the benefits of being active over a wide range of  $T_{bs}$ ? The most apparent benefit is the ability to be active during periods when ambient temperatures are relatively low. This is particularly important for temperate-zone species, such as the Grass Snake, that experience wide fluctuations in temperature ( $T_e$  range in the enclosure from May to August 2002: 10.7 °C to 70.5 °C; Mertens, 1994). This ability

to tolerate and perform at lower temperatures may allow the use of thermally marginal microhabitats, such as continuous forest (Kingsbury, 1994). By exploiting such cooler environments, Grass Snakes could gain access to different food resources.

Even in eurythermal species, however, there are limits to the range of temperatures allowing activity. These limits are principally physiological and include lower digestion rates, reduced locomotory abilities, etc. Skocylas (1970), for example, tested digestion rates of Grass Snakes over different ambient temperatures and observed that digestion was optimized over a fairly broad range of moderate to high  $T_{bs}$  (25 °C and 35 °C), but was arrested at very low  $T_{bs}$  (5 °C). Similarly, I found that Grass Snakes crawled consistently well over a wide range of higher  $T_{bs}$  (25°C to 38 °C; see Chapter 3), but performance was hindered at low  $T_{bs}$  (15 °C).

How might activity over a broad range of  $T_{bs}$  influence the fitness of Grass Snakes? In this study, I have shown that Grass Snakes maintain a wide range of  $T_{bs}$  in the field, but more important, that Grass Snakes can perform well across this temperature range (see Chapter 3). The relatively high incidence of injuries in this population suggests that encounters with predators are common, so that snakes must be able to move quickly at any  $T_{bs}$  at which they are active. However, the true fitness consequences of different thermal strategies have yet to be studied.

## LITERATURE CITED

- Autumn, K. & de Nardo, D. F. 1995. Behavioural thermoregulation increases growth rate in a nocturnal lizard. *J. Herp.* **29**(2): 157-162.
- Avery, R. A. 1982. Field studies of body temperatures and thermoregulation. In *Biology of the Reptilia* v. 12. (Gans, C. & Pough, F. H. eds.). Academic Press, Toronto. pp. 93-166.
- Bartholomew, G. A. 1986. The role of natural history in contemporary biology. *Bioscience* **36**: 324-329.
- Beebe, T. J. C. & Griffiths, R. A. 2000. *Amphibians and Reptiles*. London: Harper Collins Publishers.
- Brown, J. H. 1995. *Macroecology*. University of Chicago Press, Chicago.
- Brown, P. R. 1991. Ecology and vagility of the Grass Snake, *Natrix natrix helvetica* Lacépede. Unpubl. PhD Diss., University of Southampton, UK.
- Brattstrom, B. H. 1965. Body temperatures of reptiles. *Am. Midl. Nat.* **73**(2): 376-422.
- Burghardt, G. M. & Greene, H. W. 1988. Predator simulation and duration of death feigning in neonate Hognose Snakes. *Anim. Behav.* **36**: 1842-1844.
- Fitch H. S. 2003. Tail loss in garter snakes. *Herpetologica* **34**(3): 212-213.
- Gentilli, A. & Zuffi, M. A. L. 1995. Thermal ecology of a grass snake (*Natrix natrix*) population in Northwestern Italy. *Amphibia-Reptilia* **16**: 401-404.
- Gibson, A. R. & Falls, J. B. 1979. Thermal biology of the Common Garter Snake, *Thamnophis sirtalis* (L.). *Oecologia (Berl.)* **43**: 79-97.
- Gibson, A. R., Smucny, D. A., & Kollar, J. 1989. The effects of feeding and ecdysis on temperature selection by young Garter Snakes in a simple thermal mosaic. *Can. J. Zool.* **67**: 19-23.
- Greene, H. W. 1986. Natural history and evolutionary biology. In *Predator-Prey Relationships*. (Feder, M. E., & Lauder, G. V. eds.). Univ Chicago Press, Chicago. pp: 99-108.
- Gregory, P. T. 1977. Life-history parameters of the red-sided garter snake (*Thamnophis sirtalis parietalis*) in an extreme environment, the Interlake region of Manitoba. *Natl. Mus. Can. Publ. Zool.* **13**: 1-44.
- Gregory, P. T. & Isaac, L. A. In Press. Food habits of the Grass Snake in southeastern England: Is *Natrix natrix* a generalist predator? *J. Herp.*

- Hawley, A. W. L. & Aleksiuik, M. 1975. Thermal regulation of spring mating behaviour in the Red-Sided Garter Snake (*Thamnophis sirtalis parietalis*). *Can. J. Zool.* **53**: 768-776.
- Luiselli, L., Capula, M., & Shine, R. 1997. Food habits, growth rates, and reproductive biology of Grass Snakes, *Natrix natrix* (Colubridae) in the Italian Alps. *J. Zool. Lond.* **241**: 371-380.
- Lysenko, S. & Gillis, J. E. 1980. The effect of ingestive status on the thermoregulatory behaviour of *Thamnophis sirtalis sirtalis* and *Thamnophis sirtalis parietalis*. *J. Herp.* **14**(2): 155-159.
- Madsen, T. 1983. Growth rates, maturation and sexual size dimorphism in a population of Grass Snakes, *Natrix natrix*, in southern Sweden. *Oikos* **40**: 277-282.
- Madsen, T. 1984. Movements, home range size and habitat use of radio-tracked Grass Snakes (*Natrix natrix*) in southern Sweden. *Copeia* **1984**(3): 707-713.
- Madsen, T. 1987. Cost of reproduction and female life-history tactics in a population of Grass Snakes, *Natrix natrix*, in southern Sweden. *Oikos* **49**: 129-132.
- Madsen, T. & Shine, R. 1997. Phenotypic plasticity in body sizes and sexual size dimorphism in European Grass Snakes. *Evolution* **47**(1): 321-328.
- Mertens, D. 1994. Some aspects of thermoregulation and activity in free-ranging Grass Snakes (*Natrix natrix* L.). *Amphibia-Reptilia* **15**: 322-326.
- Peterson, C. R., Gibson, A. R., & Dorcas, M. E. 1993. Snake thermal ecology: causes and consequences of body temperature variation. In *Snakes: Ecology and Behaviour*. (Seigel, R. A. & Collins, J. T. eds.). McGraw-Hill, New York. Pp: 241-314.
- Reading, C.J. & Davies, J. L. 1996. Predation by Grass Snakes (*Natrix natrix*) at a site in southern England. *J. Zool. Lond.* **239**: 3-82.
- Rugiero, L. 1999. Death feigning in the Western Whip Snake, *Coluber viridiflavus*. *Amphibia-Reptilia* **20**(4): 438-440.
- Schaefer, W. H. 1934. Diagnosis of sex in snakes. *Copeia* **1934**: 181.
- Stevenson, R. D. 1985. The relative importance of behavioural and physiological adjustments controlling body temperature in terrestrial ectotherms. *Am. Nat.* **126**(3): 362-386.
- Stevenson, R. D., Peterson, C. R., & Tsuji, J. S. 1985. The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the Wandering Garter Snake. *Physiol. Zool.* **58**(1): 46-57.

- Van Damme, R., Bauwens, D., Brana, F., & Verheyen, R. F. 1992. Incubation temperature differentially affects hatching time, eggs survival, and hatchling performance in the lizard *Pocarcis muralis*. *Herpetologica* **48**(2): 220-228.
- Weatherhead, P. J., Barry, F. E., Brown, G. P., & Forbes, M. R. L. 1995. Sex ratios, mating behaviour and sexual size dimorphism of the Northern Water Snake, *Nerodia sipedon*. *Behav. Ecol. Sociobiol.* **56**: 301-311.

**CHAPTER 2: Thermoregulation and Thermal Limitation at a High Latitude:  
Comparison of Gravid and Nongravid Females of an Oviparous Ectotherm, the  
European Grass Snake (*Natrix natrix*)**

**INTRODUCTION**

All else being equal, when given the opportunity, ectotherms should behave so as to maintain body temperatures ( $T_b$ ; plural,  $T_b$ s) that are favourable to essential functions such as locomotion and digestion (Stevenson *et al.*, 1985; Lillywhite, 1987; Beck, 1996). Terrestrial ectotherms frequently maintain relatively constant body temperatures despite variation in environmental temperatures (Peterson, 1987; Brown & Weatherhead, 2000; Blouin-Demers & Weatherhead, 2001), although animals in different physiological states may maintain different body temperature profiles (Gibson *et al.*, 1989; Charland & Gregory, 1990).

But how common is thermoregulatory behaviour in ectotherms? Shine and Madsen (1996) argue that thermoregulation actually is unimportant for most reptiles because the majority of them live in the tropics where the environment is relatively invariant. At most, they must avoid extremely hot conditions (Akani *et al.*, 2002; Luiselli & Akani, 2002). At the other extreme, however, temperate-zone species encounter not only hot conditions during the active season, but also cool conditions. Even in hot weather, night-time temperatures often will be too low for reptiles to reach high  $T_b$ s. Thus, high-latitude environments are thermally challenging for ectotherms (Blouin-Demers & Weatherhead, 2001). 'Effective' thermoregulation (i.e.,  $T_b$ s close to preferred levels; Hertz *et al.*, 1993) may be uncommon in such environments, not because it is unimportant, but because it is difficult and potentially costly (Huey & Slatkin, 1976).

The European Grass Snake, *Natrix natrix*, is a medium-sized, oviparous, diurnal natricine snake that ranges from extreme northern Africa to near the Arctic Circle in Scandinavia (Beebe & Griffiths, 2000). Here, I present the results of a study of the thermal ecology of the Grass Snake at a high-latitude site in England. I used a semi-natural enclosure, radio-telemetry, and a novel regression analysis to address two main questions:

1. How often are environmental conditions suitable for thermoregulation? I predicted that such conditions often would be limited to mid-day, even in warm weather.
2. How well can snakes thermoregulate when conditions allow it? I predicted that, at low environmental temperatures, snakes should be thermoconformers or, at best, weak thermoregulators. Above some threshold temperature, however, snakes should be able to maintain  $T_{bs}$  that are independent of environmental temperatures.

An ancillary question that I addressed is whether gravid female snakes maintain different or less variable  $T_{bs}$  than non-gravid females. Other studies have shown this to be the case in viviparous snakes (Charland & Gregory, 1990; Charland, 1995; Brown & Weatherhead, 2000) and in at least one oviparous species (Blouin-Demers & Weatherhead, 2001). The most likely explanation for these observations is that, via careful thermoregulation, gravid females can maintain the best possible temperature for their developing offspring; thus, it should be an especially important behaviour for gravid females in cool temperate-zone sites. Oviparous snakes are still relatively unstudied in this respect and the Grass Snake, with its high-latitude distribution, is a particularly suitable study species.

## METHODS

I conducted this study during June-July 2002 in an outdoor semi-natural field enclosure at the University of Kent at Canterbury (UKC) in Canterbury, Kent, UK. The enclosure was located 3.25 km NE of my field site on the River Stour (58°N 17.58'N, 1°N 08.19'E, 5 m elevation) and thus experienced similar weather. I caught Grass Snakes opportunistically by hand and by flipping artificial cover objects. I retained gravid and nongravid females in a laboratory at UKC and chose three of each for the enclosure experiment (reduced to five snakes later- see Results). Snakes were maintained in the laboratory in individual plastic cages (45 cm X 25 cm X 25 cm) lined with newsprint. Water was provided *ad libidum* and snakes were each fed 5 goldfish approximately twice a week. I chose the largest healthy snakes in each reproductive group for implantation of 1.5 g radio-transmitters (maximum ratio of transmitter mass: body mass = 0.05:1; Model BD-2GT, Holohil Systems, Carp, Ontario, Canada; 12-week battery life at 25 °C). All animal maintenance and experimental procedures complied with guidelines for live reptiles and were approved by the University of Victoria Animal Care Committee. The pulse rate of each radio-transmitter was proportional to temperature, and calibration curves were supplied by the manufacturer (pulse rate range: 0-40 °C in 10 °C increments). I used second-order polynomial (quadratic) regressions of the 5 calibration points for each transmitter to derive an equation to predict temperature based on pulse rate. All calibration equations provided a high degree of fit ( $r^2 \geq 0.997$  in all cases).

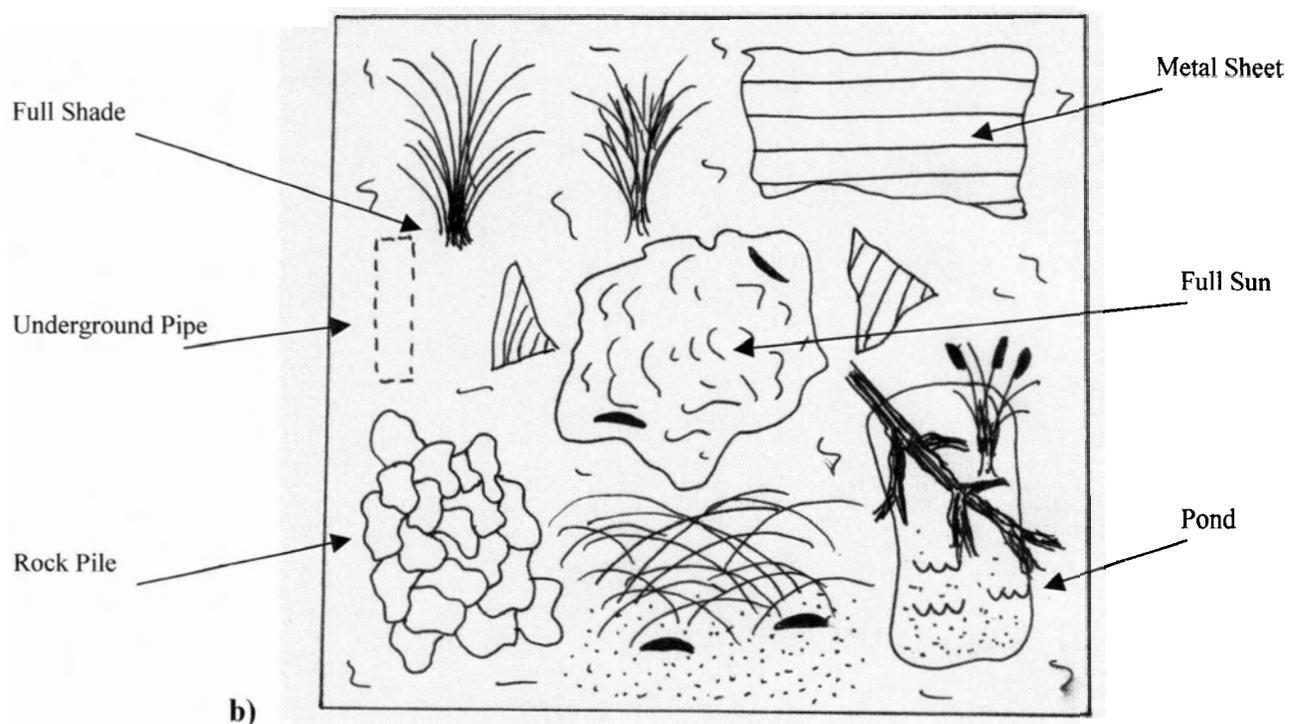
Surgery and implantation (and later removal) of transmitters were performed by a veterinary surgeon, using a modified version of Reinert and Cundall's (1982) method. Isoflurane was used to anaesthetize the snakes. Transmitters and their antennae were implanted subcutaneously with sterile techniques and 3-0 absorbable

sutures were used to close the 2 cm incision. All snakes recovered rapidly; tongue-flicking and movement resumed within 2 hours of surgery. I kept the snakes in the laboratory for 5 days before releasing them in the enclosure and did not begin monitoring  $T_b$  until 48 hours after their release.

I constructed an 8 m X 8 m enclosure at UKC in late May 2002, roughly following the design of Lee and Mills (2000). I placed the enclosure in an open area fully exposed to the sun, so that snakes would be able to achieve high  $T_b$ s in warm, sunny weather. Snakes could retreat to underground tunnels, rock crevices, and artificial cover objects for protection or cooling (Fig. 1). I also provided numerous basking sites (e.g., compost pile and bramble pile), as well as spots where snakes could raise their  $T_b$  without being exposed (e.g., under metal sheet). The vegetation was allowed to grow unhindered to provide additional cover, which I have observed snakes retreating to in the field. I created aquatic and semi-aquatic habitats by immersing vegetation into the pond and laying it on the pond edge. The 64 m<sup>2</sup> enclosure was a compromise between making construction manageable and providing sufficient room for snakes to behave naturally. I have observed Grass Snakes in close proximity to each other in the field, so I did not consider the six snakes to be too crowded, and I saw no obviously abnormal behaviour of snakes during the experiment. The enclosure simulated natural conditions as closely as possible by including a variety of microhabitats (e.g., burrows, ponds, retreat sites, basking sites) and thermal conditions (e.g., full sun, full shade, sun/shade mosaic). An artificial pond provided constant access to water and was also stocked with goldfish for food. Grass Snakes eat fish in the field (Gregory & Isaac, in press) and the laboratory (pers. obs.) and it was a convenient way to provide food.



a)



b)

**Fig. 1.** a) Photograph of the outdoor enclosure at the University of Kent at Canterbury in Canterbury, UK. b) Aerial schematic of the various microhabitats within the 64 m<sup>2</sup> enclosure.

I equipped the enclosure with 4 hollow copper-pipe models (455 mm long, 20 mm in diameter, painted grey to approximate the reflectivity of Grass Snakes) each with a thermal probe (suspended away from sides of the tube) inserted in it to record operative temperature ( $T_e$ ; plural,  $T_{e,s}$ ).  $T_e$  is the temperature that would be experienced by animals remaining in the various microhabitats in which the models were placed (Bakken, 1989). Each model, therefore, effectively gave me the temperature of a thermoconforming snake in a particular microhabitat. Effective models integrate the various heat exchange routes, such as heat conduction from the ground and absorption of solar radiation, and the general thermal qualities of the animal, such as size, shape, and reflectivity. Following this study, I compared the average spectral reflectivity of our copper-pipe model with that of a live grass snake in the laboratory using a spectrophotometer and I found some discrepancy between the two. Thus, my model could be improved for future studies. However, because temperature depends on a suite of factors, of which spectral reflectivity is only one, I nonetheless found satisfactory agreement between snake and model temperature tested in the field (see below).

I placed models in full sun, full shade, within the rock pile and under the metal sheet. I used two StowAway® TidbiT® Temp Loggers (Onset Computer Corporation, Massachusetts, USA) to characterize other microhabitats (Vitt & Sartorius, 1999); one TidbiT® was submerged in the pond and another was placed in the underground tunnel. I placed an additional TidbiT® in full sun in order to compare its temperature with that of the copper-pipe model in the same location. Although the Tidbit® differed from the shapes of both copper-pipe models and snakes, I used them simply to establish a very general picture of the thermal quality of the habitat. All snake models recorded temperature every 15 minutes.

To formally compare the temperatures of the snake models (e.g., copper-pipe model and TidbiT®) to an actual snake, I placed both models and a dead male Grass Snake (540 mm, body length) in a sunny location for a 30-hr period at the end of May 2002. The three sets of temperatures were similar except at very high levels (e.g., 40°C), at which the copper-pipe model and the TidbiT® underestimated snake  $T_b$ . To compensate for this discrepancy, I used a quadratic regression of snake temperature on model temperature to derive a 'corrected' model temperature. This 'correction' equation for the copper model provided a high degree of fit ( $r^2 = 0.983$ ). I made the appropriate adjustments to  $T_e$  measured by all snake models in all locations and used only these adjusted values in the analyses.

Grass Snakes typically are described as diurnal and night-time activity is rare except in the warmest parts of their range (Mertens, 1994). In England, nocturnal behaviour is virtually unknown (Beebe & Griffiths, 2000). Thus, I focus my analyses on daytime  $T_b$ s. I defined the daily active period as 0800-2000 and measured  $T_b$ s for each snake every 15 minutes during four 3-hr periods (0800-1100, 1100-1400, 1400-1500, 1500-2000). I measured pulses, in milliseconds, using a Pulse Interval Timer (PIT) (AVM Instrument Company Ltd., Colfax, California, USA) and converted the pulse rates to temperature. I randomly selected two of the four periods each day for these measurements. I randomly picked periods each day because thermoregulatory opportunities were highly variable and would not always occur in the same time period. Nonetheless, I constrained my selections so that each time period was represented equally often over the entire study.

Each day, I also monitored  $T_b$ s of one individual snake over a 24-hr period, using a modified automated recording system following the design of Beaupre and Beaupre (1994). I used this system to record  $T_b$  profiles during the night as well as to

evaluate the correlation between the two methods of  $T_b$  data collection. I randomized the order in which individual snakes were monitored over the study period (each snake represented equally often) and measured  $T_b$ s every 15 minutes for 24 hours starting from 0800. I determined pulse rate, in milliseconds, and converted it to temperature. I used repeated measures analysis of variance (ANOVA) to compare mean  $T_b$ s of gravid and nongravid snakes between 2000-0800 ('night'). Daytime measurements of  $T_b$  from the two collection techniques were highly correlated ( $r^2=0.98$ ;  $y=0.77 + 0.97x$ ), so I am confident that temperatures measured at night and during the day were directly comparable.

Repeated measurements of  $T_b$  for any given snake were not independent. Therefore, for analysis of daytime  $T_b$ s, I reduced serial correlation among measurements by using mean and standard deviation of  $T_b$  (and of the corresponding  $T_e$ ) as separate data points characterizing each snake's body temperature in a given time period on a given day. Because mean  $T_b$ s (and SD of  $T_b$ s) still were measured repeatedly for the same individual in different periods on different days, I constructed various repeated measures ANOVAs (repeated across time for each individual) to compare mean and standard deviation of  $T_b$  between reproductive conditions, and by time period and day.

To test for thermoregulatory behaviour, I used Huey and Slatkin's (1976) regression model, with modifications noted below. In each case, I regressed mean  $T_b$  against mean adjusted  $T_e$ . This regression should have a slope of one if animals are perfectly thermoconforming, but a slope significantly less than one if animals are thermoregulating. If thermoregulation is 'perfect' (i.e.,  $T_b$  completely independent of  $T_e$ ), then the slope of the regression should be zero. I predicted that, at lower  $T_e$ , snakes would be unable to raise their  $T_b$ s to 'preferred' levels and therefore would

either thermoconform or thermoregulate only modestly at best. At some higher  $T_e$ , however, snakes would not only be able to achieve their 'preferred'  $T_b$  but, as  $T_e$  continued to rise, would thermoregulate to avoid reaching lethal  $T_b$ .

Thus, I argue that the expected relationship between  $T_b$  and  $T_e$  should not be linear. I therefore used an alternative regression model, nonlinear piecewise regression (Neter *et al.*, 1983) to determine the  $T_b$  at which 'perfect' thermoregulation is initiated (i.e., where the slope changes). Once this point was determined, I treated the two identified 'pieces' or segments as separate linear regressions and tested them each against  $H_0$ : slope = 0 and  $H_1$ : slope = 1. I also used analysis of covariance (ANCOVA,  $T_e$  as the covariate) to compare  $T_b$ s between gravid and nongravid female snakes both above and below the change-point (preceded by test of equality of slopes).

Although a slope of 0 indicates independence of  $T_b$  from  $T_e$ , it does not necessarily represent perfect thermoregulation because variation in  $T_b$  still could be quite high (i.e., much scatter of points around the line). Furthermore, if the range of  $T_e$  is low (i.e., low variance in  $T_e$ ), evidence for thermoregulation is weak because possible  $T_b$ s could be limited by the environment. Thus, to assess precision of thermoregulation, I tested whether variance of mean  $T_b$  was significantly less than variance of mean  $T_e$  (one-tailed F-test).

I used SAS 8.0 for statistical analyses. For piecewise regression analysis, I used PROC NLIN of SAS, with the breakpoint between segments fitted as a parameter in the non-linear regression. Because of non-orthogonality of data, I used Type III sums of squares in all tests of significance. Where factors were identified as random, rather than fixed, I used the RANDOM/TEST option in PROC GLM of SAS to identify the appropriate F-tests. I considered differences to be nominally

significant at the 0.05 level. If significant effects of treatments were found, I used least-squares means (LSMEANS) to compare means from each treatment. All values are reported with mean  $\pm$  SD unless otherwise stated and the assumptions of the various analyses (normality and homogeneity of variances) were satisfied.

## RESULTS

I recorded 4,052 'manual'  $T_{bs}$  and 1,887 'telemetry'  $T_{bs}$ , ranging from 10.5 °C to 39.2 °C, from 5 snakes. In some cases, my  $T_b$  profiles were incomplete. This reflected either my inability to locate the radio-transmitter signal (due to static or temporary equipment malfunction) or radio-transmitter failure. Although snakes in the enclosure behaved in general like snakes I have observed in the field, one of the three gravid females died of unknown causes before the termination of the experiment. I therefore eliminated data from this snake from any further consideration, but dissection revealed that she apparently was resorbing her eggs when she died. Of the remaining two gravid females, only one definitely carried eggs through to oviposition; the third snake either resorbed her eggs or laid them in the enclosure before the experiment ended. Thus, on the one hand, my comparisons between gravid and nongravid female snakes are somewhat compromised. On the other hand, because of failure of one transmitter, I also restricted my analysis to just the first 33 days that the animals were in the enclosure, thus increasing the likelihood that this third snake was still carrying eggs when data were collected. Unfortunately, the transmitter that failed was carried by the one snake known to be gravid throughout the experiment, so I cannot present any definitive data from gravid females in the later stages of egg retention. However, restricting my analysis of gravid females to this one snake did not change my conclusion about comparative thermoregulatory behaviour of gravid and nongravid females.

In addition, I measured between 23,137 to 27,409 temperatures from each copper-pipe model and TidbiT® temperature recorder positioned in the various microhabitats (Table 1). In sunny weather, free-ranging Grass Snakes maintain  $T_b$ s around 30 °C (Mertens, 1994). Thus, if I use 30 °C as a preferred  $T_b$ , then opportunities to maintain preferred  $T_b$  (i.e.,  $T_e > 30$  °C) were mainly limited to midday (1100-1700) (Table 2). For preferred  $T_b$  higher or lower than 30 °C, then such opportunities were reduced or enhanced, respectively. Moreover, during these warmer periods, snakes could raise their  $T_b$ s to 30 °C or more on only 55-61 % of occasions. There were fewer opportunities to raise  $T_b$  in the morning and in the evening.

Because I frequently found wild Grass Snakes basking in the open, I assume that they are heliotherms (i.e., obtain heat energy primarily from solar radiation vs. ground) when warming. Therefore, I concluded that the appropriate  $T_e$  against which to compare daytime  $T_b$  was that of the copper-pipe model in the most exposed position in the open (i.e., the maximum available  $T_e$  at the warmest time of day). This allowed me to distinguish between behaviourally regulated versus environmentally limited  $T_b$ s. Operative temperatures measured in full sun were significantly higher ( $F_{(7, 32)} = 56.94$ ,  $P < 0.0001$ ) during the daytime periods (0800-2000) versus the evening and early morning (2000-0800) (Fig. 2). The warmest and most variable period was between 1100-1400 ( $35.4 \pm 14.5$  °C).

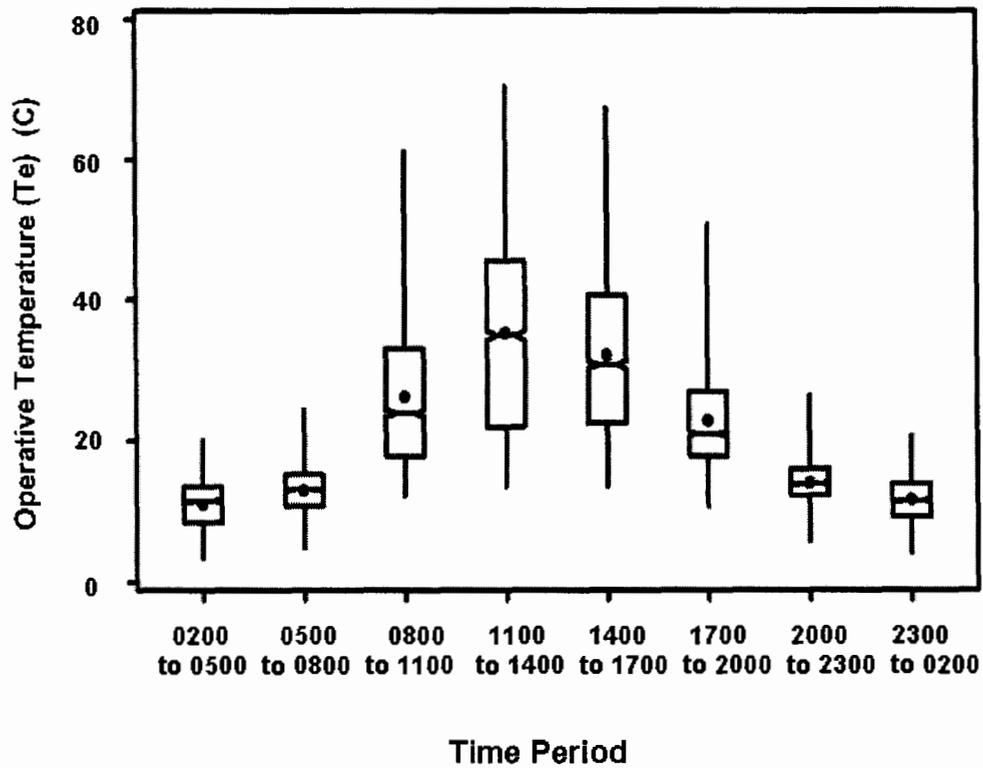
Across all days and time periods, mean  $T_b$  was significantly higher among non gravid snakes versus gravid snakes ( $25.34 \pm 5.23$  °C and  $23.12 \pm 5.91$  °C;  $F_{(1,325)} = 13.54$ ,  $P = 0.03$ ) and mean  $T_b$  was not significantly different among individual snakes nested within reproductive condition ( $F_{(3,325)} = 0.95$ ,  $P = 0.42$ ). When I included time period and day in the analysis, all main effects and the interaction between

**Table 1.** Summary of operative temperatures ( $T_{es}$ ) measured by the snake models (copper-pipe models and TidbiT®s) at various locations within the enclosure from June-July 2002. Temperatures vary by location, but overall, the warmest spot was in full sun. All  $T_{es}$  were calibrated with the snake model (see text). The discrepancy in sample size was due to equipment malfunction.

<b>Location</b>	<b><i>n</i></b>	<b>Mean (°C)</b>	<b>SD</b>	<b>Min (°C)</b>	<b>Max (°C)</b>
Full sun	23,137	21.3	12.4	3.5	70.5
Full shade	23,137	15.3	2.7	7.3	26.0
Metal Sheet	23,137	18.9	8.3	5.9	55.8
Rock Pile	23,137	16.4	3.2	9.2	31.2
Underground TidbiT®	27,409	17.2	1.8	12.5	23.0
Pond TidbiT®	27,404	19.3	3.8	11.6	31.7

**Table 2.** Summary of operative temperatures ( $T_{e,s}$ ) measured by the copper-pipe model exposed to full sun. Thermoregulatory opportunities were greatest, when  $T_{e,s} > 30$  °C, during midday. Given are the number of days and percentage of the total days when each time period met the temperature criteria. Uneven sample size is attributable to equipment malfunction.

Operative Temperature	Time of Day			
	0800-1100	1100-1400	1400-1700	1700-2000
>30	10 (37%)	17 (61%)	15 (55%)	0
20-30	11 (41%)	6 (21%)	7 (26%)	1 (4%)
<20	6 (22%)	5 (18%)	5 (19%)	23 (96%)
<b>Total Days</b>	27	28	27	24



**Fig. 2.** Box-and-whisker plot of operative temperatures ( $T_{e,s}$ ) collected over the study period that were measured by the snake model in full sun. The data are divided into eight 3-hr time periods. Thermoregulatory periods when  $T_{e,s} > 30$  °C, are limited to midday (0800-2000). Boxes represent the interquartile range (50 % of the data) divided by the median. Arithmetic mean is presented by the dot. Whiskers extend from the minimum to the maximum data values.

reproductive state and day were significant. Nongravid females maintained a consistently higher mean  $T_b$  than gravid females ( $F_{(1, 195)} = 14.37, P = 0.04$ ) throughout all time periods (reproductive state\* time period nested within day,  $F_{(33, 195)} = 0.54, P = 0.98$ ). Mean  $T_b$  also varied significantly with individual snakes, among days and between time periods nested within days. The significant interaction between reproductive state and day (i.e., the magnitude of difference between mean  $T_b$  of nongravid and gravid snakes varied among days) complicated my analysis and compromised interpretation of the main effects. Thus, I could not generalize about mean  $T_b$  over the entire study period (i.e., all days and periods).

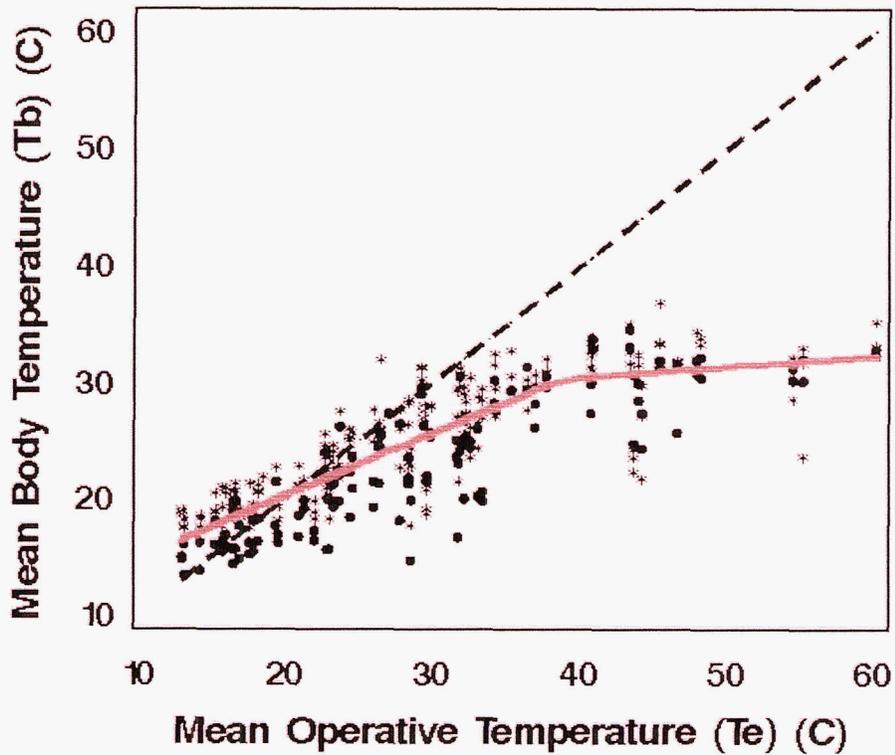
I therefore performed two simpler analyses across days and time periods. When day was removed as a factor, mean  $T_b$  of nongravid females still remained significantly higher than that of gravid females ( $F_{(1,319)} = 12.94, P = 0.04$ ) and mean  $T_b$  varied by period ( $F_{(3,319)} = 25.55, P < 0.0001$ ); mean  $T_b$  was significantly higher during midday (1100-1700) than in early morning (0800-1100) or early evening (1700-2000). When time period was removed as a factor, I observed the same significant difference in mean  $T_b$  between reproductive states ( $F_{(1,261)} = 13.54, P = 0.03$ ). Furthermore, mean  $T_b$  varied significantly by day ( $F_{(32,261)} = 18.54, P < 0.0001$ ) as well as between individuals within reproductive categories ( $F_{(3,261)} = 2.58, P = 0.05$ ). In neither of these reduced analyses were there significant interactions.

Gravid females had a higher overall mean SD than nongravid females ( $1.95 \pm 1.55$  °C &  $1.75 \pm 1.37$  °C respectively), but this difference was not significant ( $F_{(1, 195)} = 1.21, P = 0.36$ ). This pattern was constant over time period and day (i.e., non-significant interaction terms). There was significant variation in mean SD on a daily basis, in time periods within days, and between individual snakes nested within reproductive states.

For the night-time period (2000 to 0800), I compared mean  $T_b$  between individuals nested within reproductive state. Although the sample was small, nongravid females had a significantly higher mean  $T_b$  than gravid females ( $20.3 \pm 2.4^\circ\text{C}$  and  $16.6 \pm 2.4^\circ\text{C}$ ;  $F_{1, 15}=39.06$ ,  $P=0.001$ ). There was no significant difference between mean  $T_b$  of individuals within reproductive states during this period.

The change-point in piecewise regression for all individuals across days, time periods and reproductive states was at a  $T_e$  of  $38.4^\circ\text{C}$ , corresponding to a  $T_b$  of  $27.77^\circ\text{C}$  (Fig. 3). Below this temperature, the slope of the regression of  $T_b$  on  $T_e$  (0.54) was intermediate between 0 and 1 and significantly different from both (against 0:  $P<0.0001$ ; against 1:  $P<0.0001$ ;  $r^2 = 0.63$ ), suggesting that snakes were thermoregulating moderately at these lower  $T_e$ s. Above the change-point, the slope of the regression of  $T_b$  on  $T_e$  (0.09) was not significantly different from 0 ( $r^2=0.038$ ,  $P=0.09$ ) but the slope was significantly different from 1 ( $P<<0.0001$ ), providing clear evidence of thermoregulation at these higher  $T_e$ s.

Separate analyses of nongravid and gravid females showed that the  $T_e$  change-point was higher for the latter ( $37.66^\circ\text{C}$  vs.  $39.66^\circ\text{C}$ ), but this difference was non-significant (95% confidence limits:  $35.09\text{-}44.23^\circ\text{C}$  vs.  $34.00\text{-}44.33^\circ\text{C}$ ). I therefore used the common change-point indicated above in all further analyses. At high  $T_e$ s, there were no significant differences in mean  $T_b$  between reproductive states or individuals ( $F_{(1, 74)} = 0.38$ ,  $P=0.54$  and  $F_{(3, 74)} = 0.16$ ,  $P=0.92$  respectively; slopes equal), although nongravid females had slightly higher mean  $T_b$ s ( $31.1 \pm 3.4^\circ\text{C}$  vs.  $30.3 \pm 2.6^\circ\text{C}$ ). At low  $T_e$ s, there was a significant difference in mean  $T_b$  among reproductive states and individuals ( $F_{(1, 254)} = 9.28$ ,  $P=0.003$  and  $F_{(3, 254)} = 7.28$ ,  $P=0.0001$ ; slopes equal). Nongravid females had a higher mean  $T_b$  than gravid females ( $23.6 \pm 4.4^\circ\text{C}$  vs.  $21.0 \pm 4.8^\circ\text{C}$ ).



**Fig. 3.** Scatterplot of mean body temperatures ( $T_b$ s) vs. mean operative temperatures ( $T_e$ s) measured in full sun for gravid and non-gravid females during the study period. Each point represents the mean  $T_b$  for one snake in a particular time period on a particular day. The black dashed line indicates slope=1 and points falling along this line imply perfect thermoconformity (i.e.  $T_b=T_e$ ). Dots indicate mean  $T_b$ s for gravid females and stars indicate mean  $T_b$ s for nongravid females. The solid red line is the 'predicted' thermoregulatory pattern from piecewise regression for all snakes regardless of reproductive condition.

Overall, variance in mean  $T_b$  was significantly less than that of mean  $T_e$  (22.51 vs. 49.28;  $F_{(254, 254)}=2.19$ ,  $P<0.001$ ) at low  $T_{es}$  ( $T_{es}$  below the change-point). I observed similar trends at higher  $T_{es}$ , but the variance of mean  $T_b$  was markedly lower than that of mean  $T_e$  (9.89 vs. 44.74;  $F_{(74, 74)} = 4.52$ ,  $P<0.001$ ). Thus, snakes had the opportunity to maintain more variable mean  $T_b$ s when it was warmer ( $T_e$  approached 70.5 °C at one point), but instead they regulated  $T_b$ s.

## DISCUSSION

In an influential paper, Hertz *et al.* (1993) proposed a protocol for a comprehensive analysis of temperature regulation in ectotherms. Their general approach has subsequently been adopted in several studies covering various taxa (e.g., Christian & Weavers, 1996; Blouin-Demers & Weatherhead, 2001; López *et al.*, 2002; Shillington, 2002). The Hertz *et al.* protocol requires the collection of three distinct kinds of data: body temperatures ( $T_b$ s) of active animals; operative temperatures ( $T_{es}$ ) that non-thermoregulating animals potentially could experience; and the preferred or “target” body temperature range ( $T_{set}$ ) for the species. It is this last point that has generated the most significant criticism and debate (Currin & Alexander, 1999; Hertz *et al.*, 1999; Alexander & Currin, 1999). Currin and Alexander (1999) question: (1) the validity of  $T_{set}$  measured in the laboratory; (2) the assumption that  $T_{set}$  is a species constant rather than varying with the physiological state of the animal; (3) and the practice of arbitrarily representing the range of  $T_{set}$  as the central 50 % or 68 % (or some other percentage) of the data. Although Hertz *et al.*'s (1999) response does not completely obviate these and other criticisms, it does offer useful suggestions on how to deal with them.

Measurement of  $T_{set}$  is central to Hertz *et al.*'s (1993) protocol because  $T_{set}$  provides the reference point for estimation of accuracy and effectiveness of

thermoregulation. I concur that these are important aims in studies of temperature regulation in ectotherms, but my data on temperature selection by Grass Snakes in a thermal gradient suggest that it often will be difficult to determine  $T_{set}$  (see Chapter 3). I found that average  $T_b$  chosen by snakes in a gradient varied both among individuals at a given time and at different times of the active season, with no obvious ties to differences in physiological state such as age, sex or reproductive state. Thus, I cannot yet objectively define  $T_{set}$ . What then, if any, alternatives exist for analysis of thermoregulatory behaviour?

In advocating their approach to analysis of temperature regulation, Hertz *et al.* (1993) abandon Huey and Slatkin's (1976) earlier, but equally important, regression model, in which  $T_b$  is regressed on some measure of environmental temperature. A slope of 1 indicates thermoconformity, whereas a slope significantly less than 1 indicates some level of thermoregulation (independence of  $T_b$  from environmental temperature), with a slope of 0 representing complete independence. Although this approach is widely used, Hertz *et al.* (1993) rightly criticize it because most researchers have inappropriately used air (or substrate) temperature at point-of-capture as a measure of environmental temperature (e.g., Gregory, 1984). I also agree that environmental temperatures measured at point-of-capture are biased and not representative of temperatures available in the environment. In other settings, however, the regression approach can be useful, especially where movements of animals are relatively restricted and where environmental temperatures are measured by  $T_e$  independent of the snake's position, as was the case in my enclosure.

With cryptic animals such as snakes, it is important that  $T_b$  data for regression analysis be derived from radiotelemetry. "Spot"  $T_b$  measurements using thermometers can be obtained only for animals that can be caught, either on the

surface or hiding under rocks. Actively thermoregulating animals, however, might use less accessible microhabitats such as dense vegetation or underground retreats, especially in hot weather. Thus, “spot” measurements might well miss the nonlinear nature of the  $T_b - T_e$  relationship that I both predicted and found. I used a relatively novel analysis, piecewise regression, to evaluate thermoregulatory behaviour of Grass Snakes. This approach is perhaps especially appropriate for snakes that experience a wide range of  $T_e$ , including those below the range at which thermoregulation is possible. Another possibility would be to use a polynomial regression (e.g., quadratic), but this approach does not incorporate the specific predictions that I made about changes in thermoregulatory regimes with changing  $T_e$ , whereas piecewise regression does. Quadratic regression also would be more appropriate if the  $T_b$ - $T_e$  relationship followed a parabolic pattern, which clearly it does not. Simple linear regression analyses are more appropriate for more thermally benign regions with a narrower range of  $T_e$  and where the relationship between  $T_b$  and  $T_e$  can be expected to be linear (Daut & Andrews, 1993; Shine & Madsen, 1996; Akani *et al.*, 2002). My data provide clear evidence of thermoregulatory behaviour of Grass Snakes at high environmental temperatures. Furthermore, because I fit the breakpoint in the regression as a free parameter, I avoided arbitrary determination of the range of  $T_e$  over which thermoregulation is most evident.

Temperate-zone reptiles face different thermoregulatory challenges relative to reptiles inhabiting tropical regions. Ambient temperatures in the temperate zone are spatially and temporally variable, which means that reptiles will frequently be below their optimal  $T_b$ . At my site, for example, air temperatures in June and July ranged between 4.4°C and 30.8°C and averaged 15.4°C, which is typical of this region. Conversely, reptiles in tropical regions are more likely to be limited by thermally

stressful periods (i.e., reaching lethally high  $T_b$ s) rather than constrained thermoregulatory opportunities (Moore, 1978; Grant & Dunham, 1988). As expected, in this study, thermoregulatory opportunities for Grass Snakes were limited to daytime periods, as reported for other diurnally active temperate-zone species (see Brown & Weatherhead, 2000; Blouin-Demers & Weatherhead, 2001). Operative temperatures during the day (0800-2000) were highly variable (12.1°C to 70.5°C in the various microclimates) and mean  $T_e$  in full sun was relatively high ( $30.0 \pm 11.8^\circ\text{C}$ ). The warmest periods (i.e.,  $T_e > 30^\circ\text{C}$ ), however, were restricted to midday (1100-1400:  $35.4 \pm 14.5^\circ\text{C}$  and 1400-1700:  $33.2 \pm 11.6^\circ\text{C}$ ). Moreover, even during these warm periods, high mean  $T_e$ s were available on only 55-61% of the days measured.

What are the implications of a thermally limiting environment? First, opportunities for activities such as foraging and finding mates become limited because locomotory abilities are reduced at low ambient temperatures (Stevenson *et al.*, 1985; Elphick & Shine, 1998). This also will affect the animal's ability to escape predators as well as limit its reproductive output (Hawley & Aleksuk, 1974; Burger, 1998; Elphick & Shine, 1998). Second, other physiological processes such as digestion are slowed down (Stevenson *et al.*, 1985; Lillywhite, 1987), so that even if the animal can catch prey it may not be able to process it properly. Other fundamental life-history attributes also are affected because growth rates, incubation time, and embryonic viability are reduced at low temperatures (Arnold & Peterson, 1989; van Damme *et al.*, 1992; Autumn & de Nardo, 1995; Burger, 1998). From an evolutionary standpoint, if thermoregulatory costs continue to outweigh the benefits, adaptations that at least partially offset the handicap associated with a highly variable environment are expected (Autumn *et al.*, 1999). For example, we might expect the

low availability of optimal ambient temperatures to impose selection for behavioural and/or physiological adjustment to a broader and lower range of  $T_b$  for activity (Huey & Kingsolver, 1993). Previous research on Grass Snakes by Gentilli and Zuffi (1995) and Mertens (1994) shows that Grass Snakes are active across a wide range of ambient temperatures (i.e., 20.4°C to 31.5°C). Never the less, the strong relationship that I have observed in experiments assessing the relationship between locomotory performance and  $T_b$  (see Chapter 3) supports the notion that thermoregulation to an minimum optimal temperature is likely important to Grass Snakes although they are able to perform over a very broad range of  $T_b$ s.

In my study, Grass Snakes thermoregulated at all ambient temperatures during the day (i.e., all slopes <1). Thermoregulation was only moderate at lower temperatures, presumably because opportunities to raise  $T_b$  to optimal levels were limited. The benefits of thermoregulation at low  $T_e$ s can be expected to be small compared to the risks associated with activity at low temperatures. Perhaps increased wariness early in the day reflects reduced locomotory ability due to lower temperatures (Cooper, 2003); increased predation risk is therefore avoided by reduced exposure and activity. I also found significant interindividual variability at these lower temperatures; snakes may exhibit differential performance in cool conditions or this could be a statistical artefact of a small sample size. At higher ambient temperatures, variability in mean  $T_b$  among individuals disappeared and snakes maintained relatively steady  $T_b$ s well below sometimes lethally high  $T_e$ s.

Although use of variance in  $T_b$  as a measure of thermoregulatory precision is problematical in a set-point model of temperature regulation (discussion in Hertz *et al.*, 1993), I nevertheless would expect thermoregulating animals in a thermally varying environment to have less variable mean  $T_b$  than mean  $T_e$ . This was the case

in my study. Furthermore, this difference in variance was more pronounced at higher than lower  $T_{e,s}$ . Thus, although not definitive evidence of thermoregulation, these tests are consistent with my other evidence of different patterns of thermoregulatory behaviour at low and high ambient temperatures.

Why was the change-point in my regression analysis at such a high  $T_e$ ? Presumably, snakes would have been able to reach high  $T_{b,s}$  by sitting in the open when  $T_{e,s}$  were lower than 38.4°C, for example (see Gibson & Falls, 1979). However, extensive basking in the open is a risky activity, so perhaps snakes do not begin thermoregulating until they can reach optimal  $T_{b,s}$  without sitting constantly in the open (Martin & Lopez, 1999; Cooper, 2003). In my enclosure, the site with maximal potential for warming up early in the day, as measured by the copper-pipe models, was also the most exposed (compost pile in the middle of the enclosure). These results may be confounded with other ecological activities of snakes, such as feeding. For example, snakes may be passively accepting low  $T_{b,s}$  rather than actively selecting low  $T_{b,s}$  if foraging opportunities coincided with cool microhabitats. Thus, assessments of the tradeoffs between risks and benefits presumably are involved in decisions to engage in thermoregulatory behaviour (Huey & Slatkin, 1976).

Temperature shifts associated with reproductive status have been well documented in squamate reptiles (Beuchat, 1986; Charland & Gregory, 1990; Daut & Andrews, 1993; Mathies & Andrews, 1997). An upward shift in  $T_b$  of gravid females presumably increases developmental rates and shortens incubation periods, thereby reducing the period over which the potential costs of reproduction (i.e., current survival and future fecundity) are incurred. Early birth in turn would mean that temperate-zone neonates would have more time to grow and accumulate energy reserves before hibernation. Why then did gravid females in my study maintain lower

$T_b$ s than nongravid females at low  $T_e$ s? Similar trends have been observed in some lizards (Beuchat, 1986; Brana, 1993; Mathies & Andrews, 1997; Andrews *et al.*, 1997), and in one species of snake (Sanders & Jacob, 1981), although the sample size was relatively low in the latter study.

There are two possible ways to view reduced  $T_b$ s in gravid snakes: 1. lower  $T_b$ s are actively selected, or 2. lower  $T_b$ s are passively accepted. For example, if high incubation temperatures were detrimental to developing embryos, then lower  $T_b$ s would be actively selected. Alternatively, if the costs of thermoregulating (e.g., reduced locomotory ability when cool, thereby increasing predation risk) were too high, then lower  $T_b$ s simply would be accepted. Andrews *et al.* (1997) studied thermoregulatory behaviour in a sceloporine lizard and concluded that these two hypotheses (i.e., thermal stress hypothesis and encumbrance hypothesis) were complementary rather than alternative. For example, gravid females maintained lower  $T_b$ s than nongravid females when thermoregulation was easy (i.e., high ambient temperatures), presumably because high  $T_b$ s were detrimental to the developing embryos. When thermoregulation was difficult (i.e., low ambient temperatures), gravid females also maintained lower  $T_b$ s, but this time presumably because they could not move to warmer areas as easily due to their reduced locomotor ability (i.e., burden of the clutch). Seigel *et al.* (1987) showed that speed and endurance were negatively correlated with stage of pregnancy in a garter snake, but little work, to my knowledge, has focused on the effects of this factor on thermoregulatory behaviour.

I am reasonably confident that Grass Snakes in the semi-natural environment behaved 'naturally' because they exhibited behaviours similar to those of free-ranging snakes (e.g., retreating when disturbed, basking on branch piles, swimming in pond); I therefore assume that  $T_b$ s were similar to those of free-ranging snakes. Brown and

Weatherhead (2000) reported similar mean  $T_{bs}$  of Northern Water Snakes measured in captivity and in the wild. These temperature comparisons, however, can be difficult to make because controlled environments (e.g., laboratory or enclosure) can be unrepresentative of natural conditions that could affect snake behaviour. Nonetheless, I cannot completely dismiss the potential effect of the semi-natural environment on Grass Snake thermoregulatory behaviour. Further research would require replicating my experiments and increasing my sample size, in addition to complementary field studies of thermoregulation in free-ranging Grass Snakes (Mertens 1994; Gentilli and Zuffi 1995).

It was not logistically possible for me to measure a number of potentially important variables in my enclosure study. For example, although I saw snakes swimming in the pond, I did not observe actual feeding activity and could not measure food intake of snakes. Comparison of thermoregulatory behaviour of gravid and nongravid Grass Snakes therefore could have been confounded by differential feeding rates and consequent postprandial thermophily (Lillywhite, 1987; Gibson *et al.*, 1989; Beck, 1996; but see Hammerson, 1979; Brown & Weatherhead, 2000; Lysenko & Gillis, 1980). Although postprandial thermophilic responses can be of short duration (Witten & Heatwole, 1978; Gibson *et al.*, 1989), they might explain why nongravid females maintained similar, even somewhat higher,  $T_{bs}$  compared to gravid females in my study, especially if nongravid females fed more frequently than gravid (Gregory *et al.*, 1999). At my field site, in fact, stomach samples show that gravid females have food in their stomachs significantly less frequently than nongravid females (Gregory and Isaac, in press). Thus, there is a potentially complex set of interactions among reproductive state, feeding, and thermoregulation, which I could not tease apart in this

study. Further work is required to understand what role these different factors have both separately and in combination on thermoregulatory behaviour.

## LITERATURE CITED

- Akani, G. C., Eniang, E. A., Ekpo, E. J., Angelici, F. M., & Luiselli, L. 2002. Thermal and reproductive ecology of the snake *Psammophis phillipsi* from the rainforest region of southern Nigeria. *Herp. Journal* **12**(2): 63-67.
- Alexander, J. G. & Currin, S. 1999. A response to Hertz, Huey and Stevenson. *African J. Herp.* **48**(1&2): 49-51.
- Andrews, R. M. de la Cruz, F. R. M., & Santa Cruz, M. V. 1997. Body temperatures of female *Sceloporus grammicus*: Thermal stress or impaired mobility? *Copeia* **1997**: 108-115.
- Arnold, S. J. & Peterson, C.R. 1989. A test for temperature effects on the ontogeny of shape in the garter snake *Thamnophis sirtalis*. *Physiol. Zool.* **62**(6): 1316-1333.
- Autumn, K. & DeNardo, D. F. 1995. Behavioural thermoregulation increases growth rate in a nocturnal lizard. *J. Herpetol.* **29**(2): 157-162.
- Autumn, K., Jindrich, D., deNardo, D., & Mueller, R. 1999. Locomotor performance at low temperature and the evolution of nocturnality in geckos. *Evolution* **53**(2): 580-599.
- Bakken, G. S. 1989. Arboreal perch properties and the operative temperature experienced by small animals. *Ecology* **70**: 922-930.
- Beuchat, C. A. 1986. Reproductive influences on the thermoregulatory behaviour of a live-bearing lizard. *Copeia* **1986**(4): 971-979.
- Beaupre, S. J. & Beaupre, R.W. 1994. An inexpensive data collection method for temperature telemetry. *Herpetologica* **50**(4): 509-516.
- Beck, D. D. 1996. Effects of feeding on body temperatures of rattlesnakes: a field experiment. *Physiol. Zool.* **69**(6): 1442-1455.
- Beebe, T. J. C. & Griffiths, R. A. 2000. Amphibians and Reptiles. Harper Collins Publishers, London.
- Blouin-Demers, G. & Weatherhead, P. J. 2001. Thermal ecology of Black Rat Snakes (*Elaphe obsoleta*) in a thermally challenging environment. *Ecology* **82**(11): 3025-3043.
- Brana, F. 1993. Shifts in body temperature and escape behaviour of female *Podarcis muralis* during pregnancy. *Oikos* **66**: 216-222.
- Brown, G. P. & Weatherhead, P. J. 2000. Thermal ecology and sexual size dimorphism in Northern Water Snakes, *Nerodia sipedon*. *Ecol. Mono.* **70**(2): 311-330.

- Burger, J. 1998. Effects of incubation temperature of hatchling Pine Snakes: implications for survival. *Behav. Ecol. Socio.* **43**: 11-18.
- Charland, M. B. 1995. Thermal consequences of reptilian viviparity: Thermoregulation in gravid and nongravid garter snakes (*Thamnophis*). *J. Herp.* **29**(3) 383-390.
- Charland, M. B. & Gregory, P. T. 1990. The influence of female reproductive status on thermoregulation in a viviparous snake, *Crotalus viridis*. *Copeia* **1990**: 1089-1098.
- Christian, K. A. & Weavers, B. W. 1996. Thermoregulation of monitor lizards in Australia: An evaluation of methods in thermal biology. *Ecol. Mono.* **66**(2): 139-157.
- Cooper, W. E. Jr. 2003. Risk factors affecting escape behaviour by the desert iguana, *Dipsosaurus dorsalis*: speed and directness of predator approach, degree of cover, direction of turning by a predator, and temperature. *Can. J. Zool.* **81**: 979-984.
- Currin, S. & Alexander, G. J. 1999. How to make measurements in thermoregulatory studies: The heating debate continues. *African J. Herpetol.* **48**(1&2): 33-40.
- Daut, E. F. & Andrews, R. M. 1993. The effect of pregnancy of thermoregulatory behaviour of the viviparous lizard *Chalcides ocellatus*. *J. Herp.* **27**(1): 6-13.
- Elphick, M. J. & Shine, R. 1998. Long term effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards (*Bassiana duperreyi*, Scincidae). *Biol. J. Linn. Soc.* **63**(3): 429-447.
- Gentilli, A. & Zuffi, M. A. L. 1995. Thermal ecology of a Grass Snake (*Natrix natrix*) population in Northwestern Italy. *Amphibia-Reptilia* **16**: 401-404.
- Gibson, A. R. & Falls, J. B. 1979. Thermal biology of the Common Garter Snake *Thamnophis sirtalis*. I. Temporal variation, environmental effects and sex differences. *Oecologia* **43**: 79-97.
- Gibson, A. R., Smucny, D. A., & Kollar, J. 1989. The effects of feeding and ecdysis on temperature selection by young Garter Snakes in a simple thermal mosaic. *Can. J. Zool.* **67**: 19-23.
- Grant, B. W. & Dunham, A. E. 1988. Thermally imposed time constraints on the activity of the desert lizard *Sceloporous merriami*. *Ecology* **69**(1): 167-176.
- Gregory, P. T. 1984. Correlations between body temperature and environmental factors and their variations with activity in garter snakes (*Thamnophis*). *Can. J. Zool.* **62**(11): 2244-2249.

- Gregory, P. T., Crampton, L. H., & Skebo, K. M. 1999. Conflicts and interactions among reproduction, thermoregulation and feeding in viviparous reptiles: Are gravid snakes anorexic? *J. Zool. Lond.* **248**(2): 231-241.
- Gregory, P. T. & Isaac, L. A. In press. Food habits of the grass snake in southeastern England: Is *Natrix natrix* a generalist predator? *J. Herp.*
- Hammerson, G. A. 1979. Thermal ecology of the Striped Racer, *Masticophis lateralis*. *Herpetologica* **35**(3): 267-273.
- Hawley, A. & Aleksuik, M. 1975. Thermal regulation of spring mating behaviour in the Red- Sided Garter Snake (*Thamnophis sirtalis parietalis*). *Can. J. Zool.* **52**: 768-776.
- Hertz, P. E., Huey, R. B., & Stevenson, R. D. 1993. Evaluating temperature regulation by field active ectotherms: The fallacy of the inappropriate question. *Am. Nat.* **142**(5): 796-818.
- Hertz, P. E., Huey, R. B., & Stevenson, R. D. 1999. Temperature regulation in free ranging ectotherms: what are the appropriate questions? *African J. Herpetol.* **48**: 41-48.
- Huey, R. B. & Kingsolver, J. G. 1993. Evolutionary responses to extreme temperatures in ectotherms. *Am. Nat.* **141**: S21-S46.
- Huey, R. B. & Slatkin, M. 1976. Cost and benefits of lizard thermoregulation. *Quart. Rev. Biol.* **51**(3): 363-384.
- Lee, J. R. & Mills, M. S. 2000. Design and construction of an outdoor enclosure for the study of snake thermal ecology. *Herp. Rev.* **3**(1): 24-26.
- Lillywhite, H. B. 1987. Temperature, energetics and physiological ecology. In Snake: Ecology and Evolutionary Biology. (Seigel, R. A., Collins, J. T. & Novak, S. S. eds.). McGraw-Hill Publishing Company, Toronto. pp: 422-477.
- Lopez, P., Civantos, E., & Martin, J. 2002. Body temperature regulation in the amphisbaenian *Trogonophis wiegmanni*. *Can. J. Zool.* **80**: 42-47.
- Luiselli, L. & Akani, G. C. 2002. Is thermoregulation really unimportant for tropical reptiles? Comparative study of four sympatric snake species from Africa. *Acta Oecologica*, **23**: 59-68.
- Lysenko, S. & Gillis, J. E. 1980. The effect of ingestive status on the thermoregulatory behaviour of *Thamnophis sirtalis sirtalis* and *Thamnophis sirtalis parietalis*. *J. Herp.* **14**(2): 155-159.
- Martin, J. & Lopez, P. 1998. When to come out from a refuge: risk-sensitive and state dependent decisions in an alpine lizard. *Behav. Ecol.* **10**(5): 487-492.

- Mathies, T. & Andrews, R. M. 1997. Influence of pregnancy on the thermal biology of the lizard, *Sceloporus jarrovi*: Why do pregnant females exhibit low body temperatures? *Funct. Ecol.* **11**: 498-507.
- Mertens, D. 1994. Some aspects of thermoregulation and activity in free-ranging Grass Snakes (*Natrix natrix* L.). *Amphibia-Reptilia*, **15**: 322-326.
- Moore, R. G. 1978. Seasonal and daily activity patterns and thermoregulation in the Southwestern Speckled Rattlesnake (*Crotalus mitchelli pyrrhus*) and the Colorado Desert Sidewinder (*Crotalus cerastes laterorepens*). *Copeia* **1978**(3): 439-442.
- Neter, J., Wasserman, W., & Kutner, M. H. 1983. Applied linear regression models. Richard D. Irwin, Inc., Homewood, Illinois.
- Peterson, C. R. 1987. Daily variation in the body temperatures of free-ranging Garter Snakes. *Ecology* **68**(1): 160-169.
- Reinert, H. K. & Cundall, D. 1982. An improved surgical implantation method for radiotracking snakes. *Copeia* **1982**(3): 702-705.
- Sanders, J. S. & Jacob, J. S. 1981. Thermal ecology of the Copperhead (*Agkistrodon contortrix*). *Herpetologica* **37**(4): 264-270.
- Shillington, C. 2002. Thermal ecology of male tarantulas (*Aphonopelma anax*) during the mating season. *Can. J. Zool.* **80**: 251-259.
- Seigel, R. A., Huggins, M. M., & Ford, N. B. 1987. Reduction in locomotor ability as a cost of reproduction in gravid snakes. *Oecologia* **73**:481-485.
- Shine, R. & Madsen, T. E. 1996. Is thermoregulation unimportant for most reptiles? An example using Water Pythons (*Liasis fuscus*) in tropical Australia. *Physiol. Zool.* **69**(2): 252-269.
- Stevenson, R. D., Peterson, C. R., & Tsuji, J. S. 1985. The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the Wandering Garter Snake. *Physiol. Zool.* **58**(1): 46-57.
- van Damme, R., Bauwens, D., Brana, F., & Verheyen, R.F. 1992. Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. *Herpetologica* **48**(2): 220-228.
- Vitt, L. J. & Sartorius, S. S. 1999. HOBOS, TidbiTs and lizard models: the utility of electronic devices in field studies of ectotherm thermoregulation. *Funct. Ecol.* **13**: 670-674.
- Witten, G. J. & Heatwole, H. 1978. Preferred temperature of the agamid lizard *Amphibolorus nobbi nobbi*. *Copeia* **1978**: 362-364.

## CHAPTER 3: Thermal Preference and Performance of the European Grass Snake, *Natrix natrix*

### INTRODUCTION

What factors influence a terrestrial ectotherm's body temperature? Clearly, environmental temperatures will exert a large effect either directly (i.e., on reaction rates) and/or indirectly (i.e., opportunities to thermoregulate) on biological processes (Dreisig, 1980; Stevenson *et al.*, 1985; Marsh & Bennett, 1986; Gutzke & Packard, 1987; Tanaka *et al.*, 2000). Under relatively limited circumstances, however, ectotherms, such as snakes, can behaviourally regulate their body temperatures ( $T_b$ ; plural,  $T_{bs}$ ) against an environmental gradient (Stevenson, 1985).

If snakes can thermoregulate, what  $T_{bs}$  should they maintain? That is, is there a 'target' temperature or a range of temperatures at which they aim? Presumably, such a target should be a temperature range in which performance is enhanced in some way. It also follows that it should be the temperature range that snakes select when given a choice in a thermally heterogeneous environment. Temperature differences have been shown to vary with species (Lillywhite, 1987), sex (Gibson & Falls, 1979), reproductive state (Charland & Gregory, 1990), digestive condition (Beck, 1996) and season (Huey & Pianka, 1977). However, in most of these cases, optimal  $T_{bs}$  were not established.

Early studies of reptilian thermal biology suggested that snakes regulated  $T_{bs}$  around a single value, the preferred or 'target' temperature ( $T_{set}$ ), because of the unimodal frequency distributions of  $T_{bs}$  observed in the field (Brattstrom, 1965). Estimating  $T_{set}$  from field temperatures, however, is inappropriate because ecological constraints and costs are not independent of the environment, which may influence thermoregulatory behaviour (Hertz *et al.*, 1993). Body temperatures recorded from

animals held in laboratory thermal gradients may provide a more appropriate measure of  $T_{set}$  because these types of costs can be minimized. Countering this, however, is the argument that laboratory environments are not natural and therefore may lead to unnatural behaviour.

Thus, the measurement and use of  $T_{set}$ , has been debated in the literature (Currin & Alexander, 1999; Hertz *et al.*, 1999; Alexander & Currin, 1999). Currin and Alexander (1999) question not only the validity of  $T_{set}$  because it is measured in an artificial laboratory setting, but the assumption that  $T_{set}$  is a species constant rather than varying with the physiological state of the animal (Paterson & Davies, 1978; Gibson *et al.*, 1989). Reference to a single  $T_{set}$  will sometimes be inappropriate and caution must be exercised when interpreting such results (i.e., integrating field and laboratory data). However, laboratory tests represent a logical starting point for studies of this type.

Do snakes actually choose  $T_{bs}$  that coincide with maximum performance? Few studies have addressed the correlation between performance and temperature choice. The best example is Stevenson *et al.* (1985), who showed that garter snake performance was enhanced at higher  $T_{bs}$ , which coincided with warmest daytime periods for activity. Here I compare performance and temperature choice in a high-latitude natricine snake, the Grass Snake, *Natrix natrix*, an ecologically similar relative of garter snakes (*Thamnophis* sp.). *Natrix natrix* occurs across a wide range of latitudes, approaching the Arctic Circle in Scandinavia. In northern parts of its range, it thus encounters highly variable thermal environments and therefore might be expected to have relatively broad thermal preferences and performance optima. I address three questions:

1. How does performance vary with temperature? I tested crawling speed, swimming speed, and tongue-flick rate over a broad range of  $T_{bs}$ .
2. What  $T_{bs}$  do snakes choose in a laboratory gradient (i.e.,  $T_{set}$ )? Do they correspond closely with  $T_{bs}$  for maximum performance?
3. How often can  $T_{set}$  be achieved in the field? That is, to what extent are optimal temperatures feasible?

## METHODS

I conducted this study during July-August 2002 at the University of Kent at Canterbury (UKC) in Canterbury, Kent, UK. The laboratory was located approximately 3.25 km NE from my field site on the River Stour (58°N 17.58'N, 1°N 08.19'E, 5 m elevation) where I caught Grass Snakes opportunistically by hand. I maintained nongravid females and males at UKC in individual plastic cages (45 cm X 25 cm X 25 cm) lined with newsprint. Water was provided *ad libitum* and snakes were each fed five goldfish approximately twice a week. Male Grass Snakes ranged in size from 480 to 721 mm body length and females ranged in size from 650 to 958 mm body length.

I tested the relationship between crawling speeds and temperature using data from two separate experiments. In the first experiment, I constructed a 2.0 m long, 0.5 m wide and 0.5 m high racetrack out of cardboard. To measure crawling speeds, I chased 25 snakes down the racetrack while continuously tapping their tails to stimulate maximum movement down the track. I measured each trial using a digital stopwatch and then converted each time to speed (m/sec). I repeated this three times for each snake at each of three body temperatures ( $T_{bs}$ ): 16 °C ('cool'), 21 °C ('room'), and 32 °C ('warm'). Snakes were given a one hour rest between trials and I did not race snakes that had recently eaten.

The data for the second experiment were collected by P. T. Gregory in May 2003. Temperatures used in this experiment ranged from 25 °C ('room') to 38 °C ('very hot'). Thus, these data complemented my data and allowed me to construct a more complete performance curve. Five snakes from the same Grass Snake population in Fordwich, UK were used and crawling tests were conducted at UKC. Crawling speed was measured in a similar fashion (i.e., movement was stimulated), although the experimental design differed somewhat in time of year (May vs. August), substrate type (cardboard vs. linoleum), and track length (1 m vs. 2 m track). Nonetheless, I can make general comparisons using the 2 sets of data.

I modified a plastic drainage pipe to construct a 4.0 m long, 0.5 m wide and 0.5 m high swimtrack. I used a longer swimtrack (4 m vs. 2m crawling track) because the snakes could swim the shorter track very quickly (approximately 2-3 seconds) and experimental error was a concern. I kept water in the trough at approximately 0.2 m depth and 20 °C. To measure swimming speeds, I chased 28 Grass Snakes down the swimtrack, tapping their tails continually to stimulate maximum movement. Snakes did not appear to acclimatize to being chased. I measured each trial using a digital stopwatch and then converted each time to speed (m/sec). I repeated this three times at each of three  $T_b$ s: 13 °C ('cool'), 21 °C ('room'), 34 °C ('warm'). I rested snakes for one hour between subsequent trials and I did not race snakes that had recently eaten.

If a snake balked while crawling or swimming by either refusing to move down the track or by reversing direction, the trial was stopped and immediately restarted (within 10 seconds). If this behaviour was repeated more than two times, the snake was taken out of rotation and re-run at the end of the trial. Mean and maximum speeds were calculated for the crawling and swimming experiments only if at least

two of the three trials at a particular  $T_b$  were completed (hence smaller sample sizes in some cases).

After each swimming trial, each snake was hand-held in front of a Sony DCR-TRV10E (1999) digital videorecorder for a 30-second interval to video-record tongue flick frequency. I analyzed each videocassette tape using a Sanyo (VHR H600 model) video cassette player. Mean and maximum frequencies were calculated for each snake and individuals that did not flick their tongues in a particular trial were given a count of '0'.

In each experiment, I controlled  $T_b$ s by placing each animal in either a constant temperature cooler or incubator approximately 30 minutes before each trial. In crawling speed trials by P. T. Gregory, snakes were maintained in a constant-temperature incubator for approximately 40 minutes before each trial. Before each snake was tested, I measured cloacal temperatures using an Omega Digital Thermometer (Model: HH82; Omega Engineering Stamford, CT). Because of limited space and equipment, all of the experiments were conducted in a laboratory where ambient temperatures could not be controlled causing  $T_b$ s to shift. I disregard this as a potential confounding factor because trials were completed quickly.

I created a thermogradient ranging from 10 °C to 50 °C in a 100 cm X 40 cm X 30 cm glass terrarium. I provided continuous cover throughout by affixing stiff paper 10 cm above the floor. Between trials, I thoroughly cleaned the gradient to eliminate any snake odours. I randomly chose three of the largest, healthiest, nongravid Grass Snakes to be tested separately in the gradient (i.e., 1 trial: 1 snake). I did not feed snakes for at least seven days prior to trials because I wanted to control for possible postprandial effects (Kitchell, 1969; Beck, 1996). The experiment was done twice (June and August) using the same snakes each time. I used temperature-

sensitive transmitters (1.5 g, maximum ratio of transmitter mass: body mass = 0.05:1; Model BD-2GT, Holohil Systems, Carp, Ontario, Canada; 12-week battery life at 25 °C) to measure  $T_{bs}$ . I force-fed one transmitter to each snake approximately 36 hours before each trial. I allowed each snake to acclimatize to the terrarium for 24 hours before I began monitoring  $T_{bs}$ . Water was provided *ad libitum* during the pre-experimental period but was removed at the onset of the experiment. I monitored  $T_{bs}$  every 15 minutes over a 24-hr period using a modified automated recording system following the design of Beaupre and Beaupre (1994). The pulse rate of each transmitter was proportional to temperature and calibration curves were supplied by the manufacturer (pulse rate range: 0-40 °C in 10 °C increments). I used second-order polynomial (quadratic) regressions of the five calibration points for each transmitter to derive an equation to predict temperature based on pulse rate. All calibration equations provided a high degree of fit ( $r^2 \geq 0.997$  in all cases).

Because speeds and tongue flick frequencies were measured repeatedly for the same individual over different temperatures, the data were not statistically independent. I therefore used univariate repeated measures analysis of variance (ANOVA) models (individuals nested within sex) to compare mean and maximum speeds between individuals, sexes and  $T_{bs}$ . I analyzed the two crawling speed experiments separately.

Repeated measurements of  $T_{bs}$  of snakes in the thermogradient also were not independent. I therefore reduced serial correlation among measurements by using mean  $T_b$  in each of three 8-hr time periods (0900-1700, 1700-0100, 0100-0900) per day. In total, I had 3 data points per snake for each of two periods (June and August) ( $n = 18$ ). I constructed two 1-way ANOVA models with  $T_b$  as the dependent factor and individual as a random factor to compare each snake's preferred body

temperature pattern in each time period. There are different ways to calculate  $T_{set}$ , some of which include the mean, the median, or the central 50 % or 68 % of all  $T_b$ s selected in the laboratory (Hertz *et al.*, 1993). I adopted two approaches in my analyses. My first estimate of  $T_{set}$  was the most conservative because it was the lowest temperature selected in the thermogradient. For my second estimate, I calculated mean  $T_{set}$  for each snake across months. I arbitrarily used the lowest mean of these three values as the second estimate of  $T_{set}$ . I compared these values (28.6 °C and 38.4 °C respectively) to temperatures measured for active animals caught in the field. I used a quick-read digital thermometer inserted in the cloaca to measure  $T_b$ s immediately after capture and I used physical models (mercury thermometer in metal case) to measure operative temperatures ( $T_e$ ). The thermometer was placed in the exact location where the snake was located and allowed to equilibrate for at least 1 minute before I took the measurement.  $T_e$ s are temperatures that would be experienced by animals remaining in the various microhabitats in which the models were placed; collectively,  $T_e$ s provide a measure of potential  $T_b$ s available in the field (Bakken, 1989).

The use of field temperature data for evaluating  $T_{set}$  is questionable because these measurements are restricted primarily to ‘active’ animals. In order to evaluate  $T_{set}$  opportunities over a wider range of conditions and over a longer span of time, I used  $T_e$  data collected following spot temperature measurements of snakes captured in the field as well as  $T_e$  data collected over a 2-month period in a semi-natural enclosure. I placed 6 snake models (i.e., copper pipe models) in various microhabitats (e.g., full sun, full shade, rock pile, pond, underground tunnel and metal sheet) throughout the enclosure and recorded  $T_e$ s every 15 minutes.

I used SAS 8.0 for statistical analyses. Because of non-orthogonality of data, I used Type III sums of squares in all tests of significance. Where factors were identified as random (e.g., individual snakes), rather than fixed, I used the RANDOM/TEST option in PROC GLM of SAS to identify the appropriate F-tests. I considered differences to be nominally significant at the 0.05 level. If significant effects of treatments were found, I used least-squares means (LSMEANS) to compare means from each treatment. All values are reported with mean  $\pm$  SD and the assumptions of the various analyses (normality and homogeneity of variances) were satisfied.

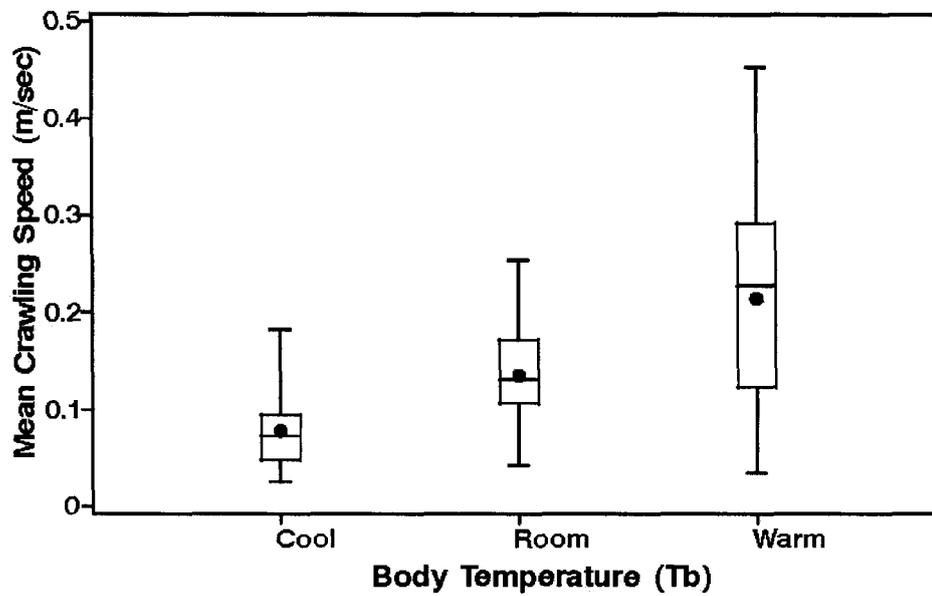
All animal maintenance and experimental procedures complied with guidelines for live reptiles and were approved by the University of Victoria Animal Care Committee.

## RESULTS

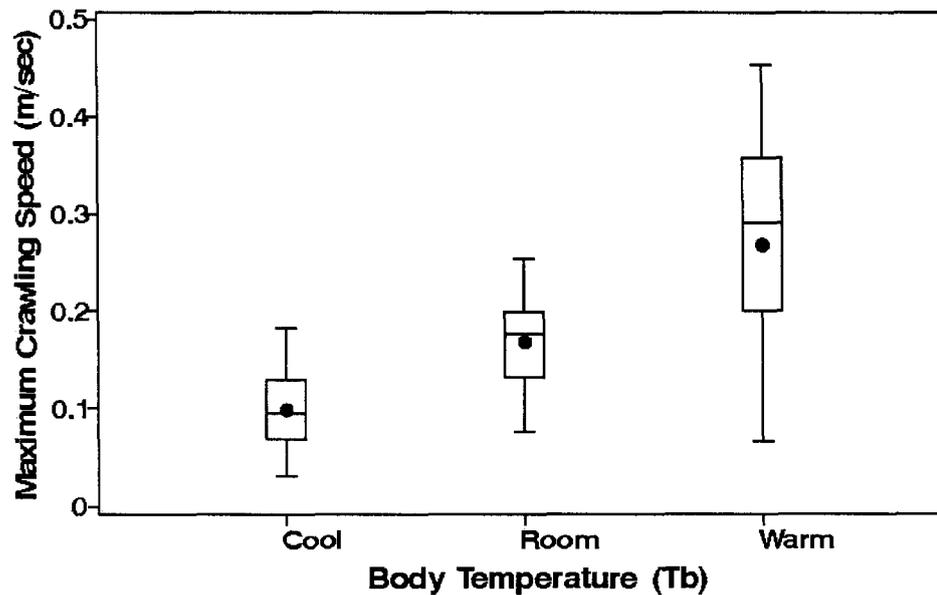
In analyses for all experiments, males and females were combined because performance did not vary with sex and the change in performance with temperature varied consistently with sex (i.e., temperature\*sex interaction always nonsignificant).

In the first crawling experiment, mean crawling speed differed among individuals ( $F_{(25,185)}= 6.89, P<0.0001$ ) and with body temperatures  $T_{bs}$  ( $F_{(2,185)}= 122.94, P<0.0001$ ). Mean crawling speed was significantly higher with higher  $T_{bs}$  (Fig. 1). Maximum crawling speed also differed significantly among individuals ( $F_{(25,43)} 3.36, P=0.0002$ ) and with  $T_{bs}$  ( $F_{(2,43)}= 60.06, P<0.0001$ ) and with  $T_{bs}$ . Maximum crawling speed was significantly high with higher  $T_{bs}$  (Fig. 2).

In the second crawling experiment, mean crawling speed differed among individuals ( $F_{(4,52)}= 14.20, P<0.0001$ ) and with  $T_{bs}$  ( $F_{(3,52)}= 3.68, P=0.02$ ). The only significant differences in mean speed, however, were between 25 °C and 30 °C



**Fig. 1.** Boxplot of mean crawling speeds (m/sec) measured at three different body temperatures (cool: 16 °C, room: 21 °C, warm: 30 °C). Mean crawling speed increases with rising body temperature. Boxes represent the interquartile range (50 % of the data) divided by the median. Arithmetic mean is presented by the dot. Whiskers extend from the minimum to the maximum data values.



**Fig. 2.** Boxplot of maximum crawling speeds (m/sec) measured at three different body temperatures (cool: 16 °C, room: 21 °C, warm: 30 °C). Maximum crawling speed increases with rising body temperature.

( $P=0.02$ ) and 25 °C and 34 °C ( $P=0.03$ ). Above 30 °C, however, speed levelled off (at 34 °C) and decreased slightly but nonsignificantly (at 38 °C; Fig. 3).

Maximum crawling speed also significantly differed among individuals ( $F_{(4,12)}= 5.53$ ,  $P=0.009$ ) but not with  $T_b$  ( $F_{(3,12)}= 1.60$ ,  $P=0.24$ ) (Fig. 4). Maximum crawling speed did not differ significantly between any of the temperature treatments.

Similar patterns emerged from the swimming experiments: mean swimming speed differed among individuals, although this difference was not as strong as in the crawling experiments ( $F_{(27, 207)}= 4.39$ ,  $P<0.0001$ ). Mean swimming speed increased significantly with increasing  $T_b$  ( $F_{(2, 207)}=63.48$ ,  $P<0.0001$ ) and mean speed was significantly different at each temperature (Fig. 5). Maximum swimming speed differed significantly both with individuals ( $F_{(27, 52)}=1.81$ ,  $P=0.03$ ) and at each temperature ( $F_{(2, 52)}=21.91$ ,  $P<0.0001$ ) (Fig. 6).

There was a significant difference in mean tongue flick frequency between individuals ( $F_{(27,216)}= 6.68$ ,  $P<0.0001$ ) and with temperature ( $F_{(2,216)}= 16.05$ ,  $P<0.0001$ ). Mean tongue flick frequency, however, did not increase with rising body temperature, but was greatest at low temperatures (Fig. 7). Similar trends emerged for maximum tongue flick frequency: individuals differed significantly ( $F_{(27,53)}= 3.16$ ,  $P=0.0002$ ) and maximum frequency differed with  $T_b$  ( $F_{(2,53)}= 3.50$ ,  $P=0.04$ ; Fig. 8). Maximum frequency was greatest when snakes were coolest and there were small differences in frequency between the 'room' and 'hot'  $T_b$ s. Variation in mean and maximum frequency was smallest at low temperatures.

I determined the thermal preference of Grass Snakes from 533 temperature measurements. Overall, actual body temperatures ( $T_b$ s) ranged from 28.6 °C to 42.7°C and mean  $T_b$ s varied from 34.8 °C to 39.6 °C between individuals. Snakes

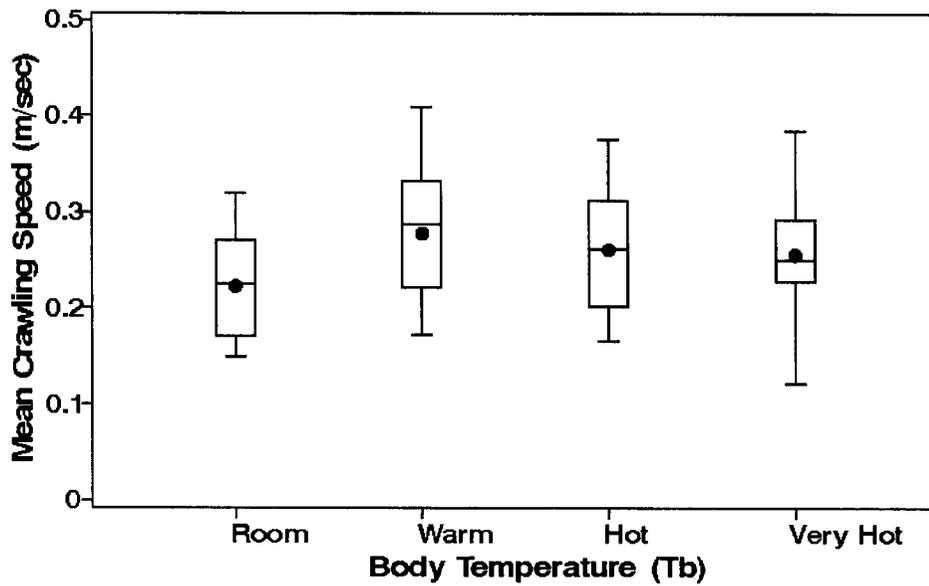


Fig. 3. Boxplot of mean crawling speeds over a higher range of body temperatures (room: 25 °C, warm: 30 °C, hot: 34 °C, very hot: 38 °C). Mean crawling speed increased slightly, levelled off, and was followed by a nonsignificant reduction in speed. Data collected by P. T. Gregory.

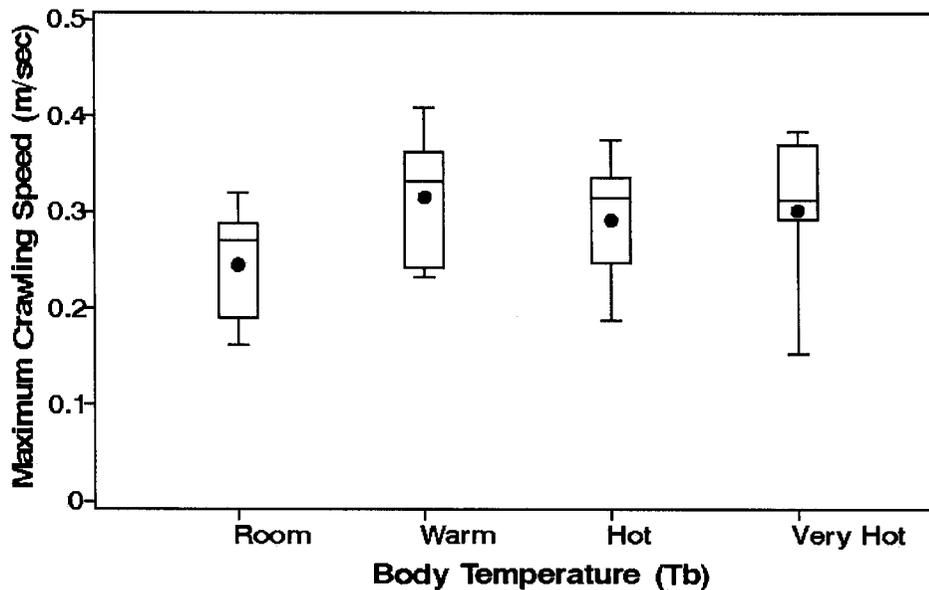


Fig. 4. Boxplot of maximum crawling speeds over a higher range of body temperatures (room: 25 °C, warm: 30 °C, hot: 34 °C, very hot: 38 °C). Maximum crawling speed did not vary significantly with temperature. Data collected by P. T. Gregory.

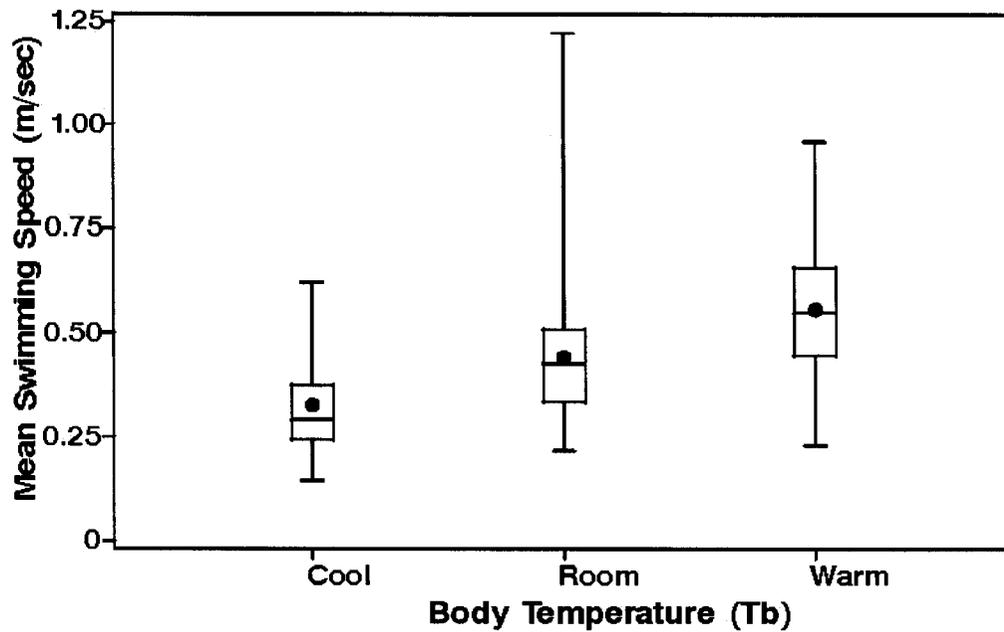


Fig. 5. Boxplot of mean swimming speeds (m/sec) measured at three different body temperatures (cool: 16 °C, room: 21 °C, warm: 30 °C). Mean swimming speed increases with rising body temperature.

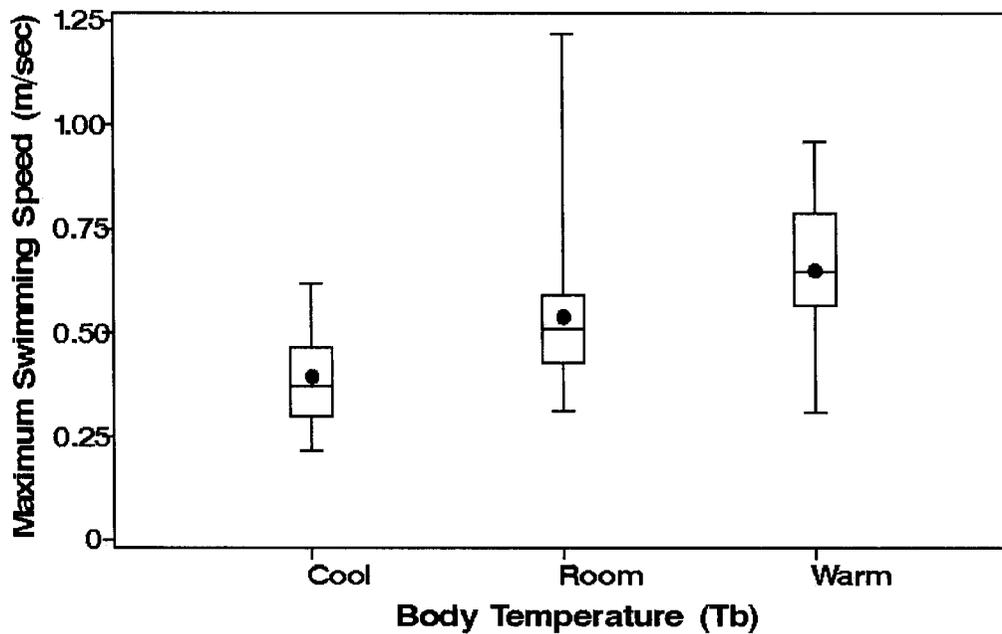


Fig. 6. Boxplot of maximum swimming speeds (m/sec) measured at three different body temperatures (cool: 16°C, room: 21°C, warm: 30°C). Maximum swimming speed increases with rising body temperature.

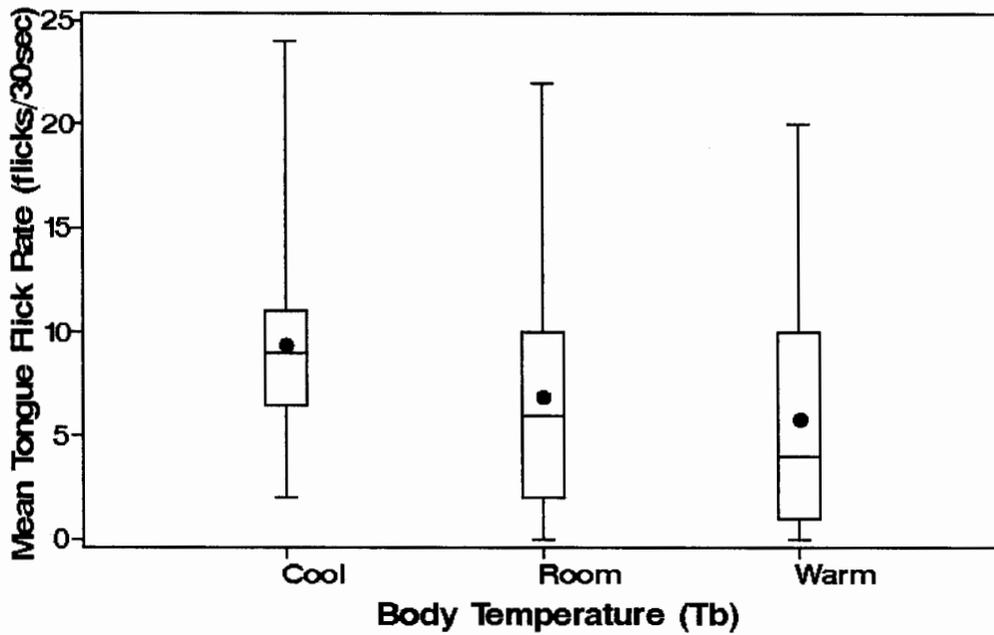


Fig. 7. Boxplot of mean tongue flick frequency over a 30-sec interval at different body temperatures. Mean frequency is greater at lower temperatures.

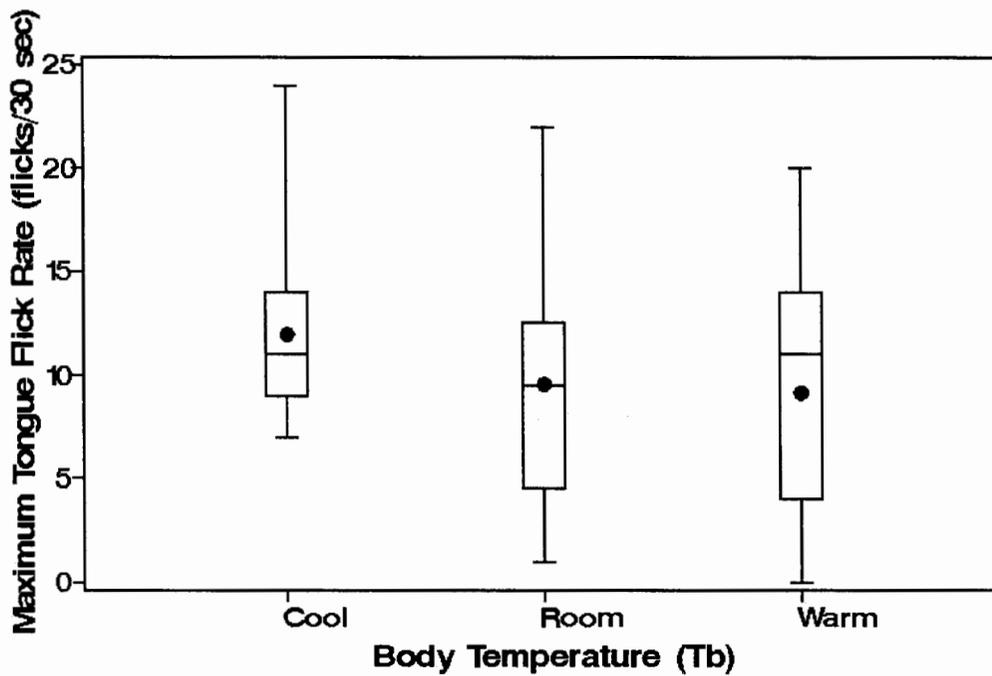


Fig. 8. Boxplot of maximum tongue flick frequency over a 30-sec interval at different temperatures. Maximum frequency is greater at lower temperatures.

maintained high  $T_{bs}$  and the grand mean for all snakes across both months was  $36.4 \pm 2.9$  °C.

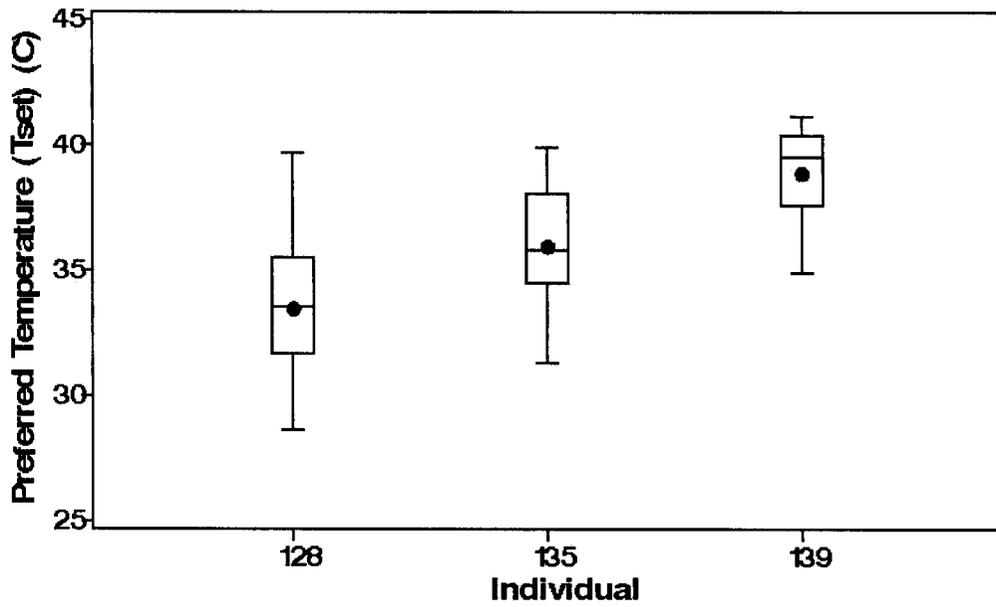
There were no significant interactions between either individuals and time periods ( $F_{(4, 6)} = 0.29$ ,  $P=0.88$ ) or individuals and months ( $F_{(2, 6)} = 3.41$ ,  $P=0.10$ ), so these interactions were deleted from the analyses. Body temperatures were somewhat lower in June than in August (June:  $36.0 \pm 3.0$  °C, August:  $37.0 \pm 2.9$  °C), although this difference was nonsignificant ( $F_{(1,12)} = 1.09$ ,  $P=0.32$ ) (Fig. 9a, b).

There was a significant difference in mean  $T_{bs}$  maintained by individual snakes ( $F_{(2,12)} = 13.05$ ,  $P=0.001$ ). One snake (#139) maintained consistently higher  $T_{bs}$  ( $39.59 \pm 1.4$  °C vs.  $34.8 \pm 1.7$  °C and  $35.0 \pm 1.7$  °C).

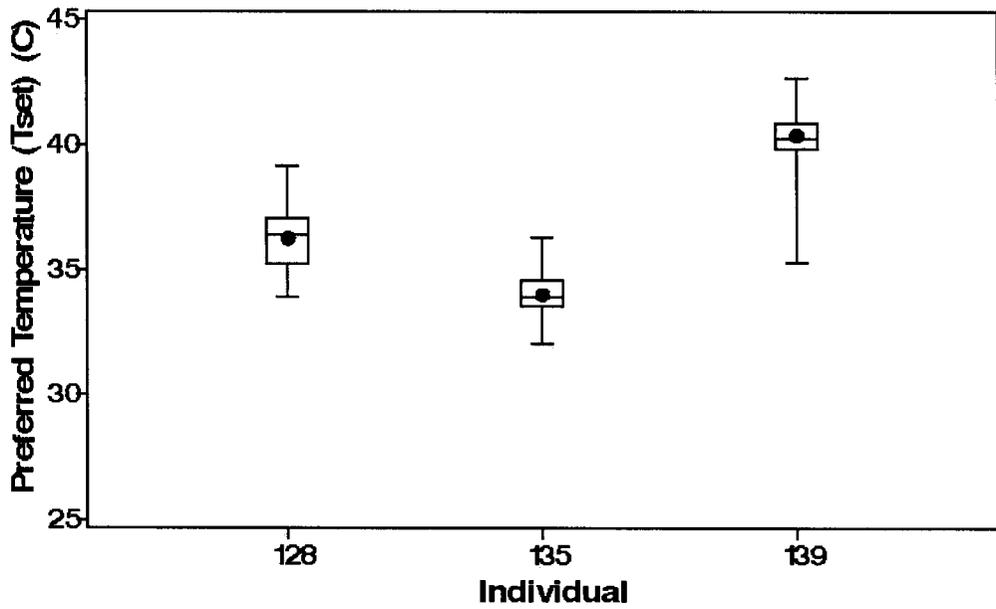
I collected operative temperature ( $T_e$ ; plural,  $T_{es}$ ) data for 42 snakes captured in the field. Of those captures, only seven snakes had  $T_{es} \geq 28.6$  °C. I recorded only one snake where  $T_e > 38.4$  °C. In the outdoor enclosure, opportunities for snakes to achieve high  $T_{bs}$  (i.e.,  $T_{es} > 28.6$  °C) were greater in the day (0800-2000) than at night (2000-0800) (Table 1 a, b). Some microhabitats were warmer than others (Fig. 10 a-f). I recorded  $T_{es}$  of at least 28.6 °C in the pond and in the rock pile on limited occasions (i.e., afternoon and early evening) (Table 2). I did not record  $T_{es} \geq 38.4$  °C in these locations at any time.  $T_{es}$  greater than 38.4°C were abundant in full sun and under the metal sheet. High temperatures could be attained most often in full sun (Table 2).

## DISCUSSION

Although temperature influences behavioural and physiological functions in reptiles, individuals can be active over a wide range of body temperatures ( $T_{bs}$ ) (Stevenson, *et al.*, 1985; Kingsbury, 1994; Manning & Grigg, 1997). Maximal



a)



b)

**Fig. 9.** Boxplot of body temperatures selected ( $T_{set}$ ) by three snakes in a thermogradient in a) June, 2002, and b) the same three snakes in a thermogradient in August, 2002.

**Table 1.** a) Summary of operative temperatures ( $T_{es}$ ) measured in various locations throughout the outdoor enclosure during the day (0800-2000). Snakes could reach high  $T_{bs}$  (i.e.  $T_{es} \geq 28.6$  °C) in 4 of the 6 locations (\*). b) Summary of  $T_{es}$  measured in the same locations during the night (2000-0800). Snakes had no opportunities to raise their  $T_{bs}$  to high levels (i.e.  $T_{es} \geq 28.6$  °C) in any microhabitat.

a)

Location	n	Mean	SD	Min	Max
* Full Sun	2042	29.1	12.2	10.7	70.5
* Metal Sheet	2042	24.1	8.1	12.3	55.8
* Rock Pile	2042	17.9	3.4	11.5	30.2
* Pond	2420	20.7	4.0	12.6	55.8
Full Shade	2042	16.7	2.5	11.9	26.0
Underground	2420	17.7	1.8	13.1	22.5

b)

<b>Location</b>	<b>n</b>	<b>Mean</b>	<b>SD</b>	<b>Min</b>	<b>Max</b>
Full Sun	1815	12.5	3.4	3.4	26.5
Metal Sheet	1815	13.0	2.5	5.9	21.5
Rock Pile	1815	14.7	2.1	9.2	23.4
Pond	2149	17.7	2.7	11.6	26.7
Full Shade	1815	13.6	1.7	7.3	19.6
Underground	2149	16.6	1.5	12.5	21.4

**Table 2.** Summary of the number of daytime periods when snake  $T_{bs}$  could reach at least 28.6 °C and 38.4 °C (two estimates of  $T_{set}$ , see text). Shown are the warmest microhabitats measured in the outdoor enclosure.  $T_{set}$  could be achieved more frequently in full sun.

**28.6°C**

Location	0800-1100	1100-1400	1400-1700	1700-2000
<i>Full Sun</i>	26	33	33	23
Metal Sheet	18	28	24	6
Rock Pile	0	0	1	1
Pond	0	1	3	1

**38.4°C**

Location	0800-1100	1100-1400	1400-1700	1700-2000
<i>Full Sun</i>	18	27	23	9
Metal Sheet	4	15	12	0
Rock Pile	0	0	0	0
Pond	0	0	0	0

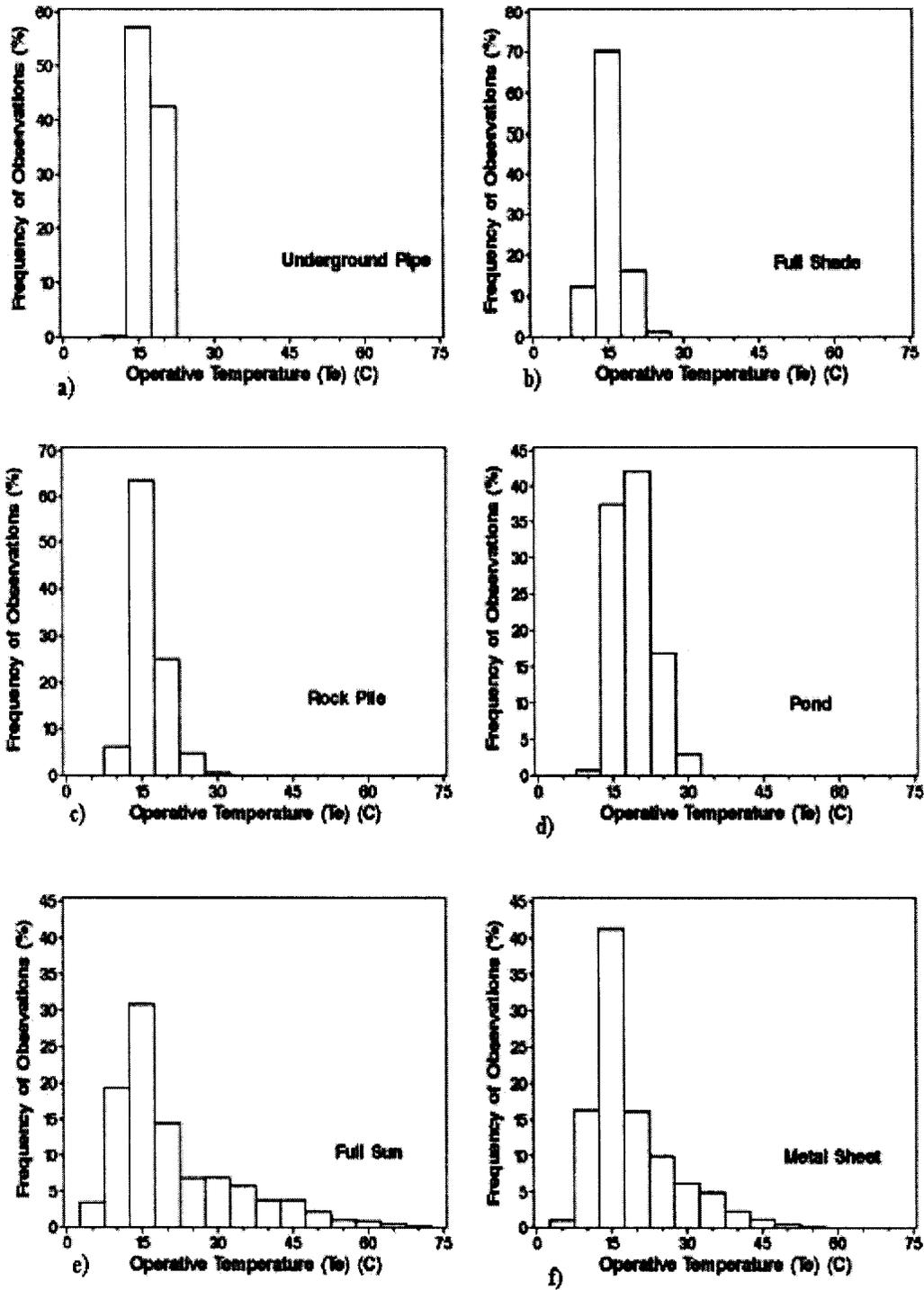


Fig. 10. Histogram of operative temperatures ( $T_e$ s) measured in a) in an underground pipe, b) in full shade, c) in a rock pile, d) in the pond, e) in full sun and f) under a metal sheet. Note different scales in some of the graphs.

study, there were significant interindividual differences in performance. All snakes, however, performed over a wide range of  $T_{bs}$  (13 °C to 38 °C).

Both crawling and swimming velocities increased with rising  $T_{bs}$ ; however, crawling performance remained stable at high  $T_{bs}$  (30 °C to 34 °C) and decreased slightly only at extremely high  $T_{bs}$  (38 °C). Although swimming performance at high  $T_{bs}$  was not tested here, similar patterns have been observed in other reptiles (Hailey, 1984; Stevenson *et al.*, 1985; Weatherhead & Robertson, 1991; Elsworth *et al.*, 2003). Threshold or lethal temperatures set limits on performance because whole body functions beyond these critical values are affected (Huey, 1982; Bennett, 1985). Although I did not observe this in my experiments, presumably this decline at high temperatures is in response to impairment of body function as  $T_{bs}$  reach critical maximums (Bennett, 1985).

What are the implications of locomotion that is thermally dependent? First, because warmer animals generally move faster, these individuals presumably have a better chance of escaping predation and avoiding injury or death. Thermal conditions, however, affect anti-predator responses differently (e.g., approach distance, flight distance, time spent in refuges and defensive displays) with long-term consequences for fitness (Christian & Tracy, 1981; Burger 1998). Second, activity levels presumably also increase with rising  $T_{bs}$  (Grant & Dunham, 1988), increasing encounter rates with prey. Striking velocity and capture success increase at high  $T_{bs}$  (Greenwald, 1974), with implications for growth rates (Dunham, 1978).

In contrast to locomotion, tongue flick rate was negatively related to temperature. Why this should be is not clear, but there are at least two possible, and very different, interpretations: 1. my experimental design was faulty, and, 2. there are advantages to higher tongue flick rates at lower temperatures. The latter, however,

contradicts previous studies (Stevenson *et al.*, 1985; Cooper & Vitt, 1986). My experimental design is questionable because snakes were probably not being tested at the appropriate temperatures. Because the tongue-flicking trials were performed after the swimming trials, there is a high probability that  $T_{bs}$  had either decreased or increased. However, if my results are in fact accurate, perhaps this performance trend is an illustration of a biological function that is important at low  $T_{bs}$ . Different performance functions are optimized at different  $T_{bs}$ , suggesting that no single  $T_b$  is ideal for every ecological situation. In this example, low  $T_{bs}$  may hinder locomotory abilities of Grass Snakes, but higher tongue flick rates associated with low  $T_{bs}$  may be important in obtaining information about an animal's environment when risks are high (e.g., if snakes have a low chance of escaping from a predator). Other reptiles, such as lizards, employ other behaviours such as posturing, vocalizing, and biting when locomotory abilities are impaired at low  $T_{bs}$  (Hertz *et al.*, 1982; Crowley & Pietruszka, 1983).

Generally speaking, Grass Snakes maintained  $T_{bs}$  in the thermogradient ( $T_{set}$ ) that were relatively high compared to other temperate-zone snakes (Grass Snakes  $36.4 \pm 2.9$  SD °C, this study; Water Snakes  $27.1 \pm 2.43$  SD °C, Brown & Weatherhead, 2000; Garter Snakes  $29.6 \pm 0.25$  SE °C, Peterson, 1987; Rat Snakes 28.1 °C, Blouin-Demers & Weatherhead, 2001). The high  $T_{set}$  selected by Grass Snakes is consistent with optimal  $T_{bs}$  for locomotory performance. The broad range of  $T_{bs}$  over which Grass Snakes maximize crawling speeds matches my initial predictions. Snakes rarely achieve very high  $T_{bs}$  in the field. Temperatures at which they thermoregulate ( $T_{bs}=27.7$  °C) are nonetheless within the lower end of the range of  $T_{bs}$  at which performance is optimized.

My results must be interpreted with caution because of a number of experimental issues. Crawling performance of Grass Snakes was probably conservative because it was measured on a slippery substrate and probably not a true reflection of speed possible in the field. Also, I tested crawling ability on a 2 m track, which perhaps better tested endurance than maximum speed. The swimtrack also may have been too narrow, restricting lateral swimming motion, but the size was a compromise between materials available and the maximum size that I could safely retrieve the animals out of the container without escaping. A variety of different sized swimtracks would be needed in order for this to be tested. However, even if the experimental design did not allow speeds as high as those possible in the field, this should not have influenced my comparisons between different temperatures, the main point of these tests.

## LITERATURE CITED

- Alexander, J. G. & Currin, S. 1999. A response to Hertz, Huey and Stevenson. *African J. Herp.* **48**(1&2): 49-51.
- Beck, D. D. 1996. Effects of feeding on body temperatures of rattlesnakes: A field experiment. *Physiol. Zool.* **69**(6): 1442-1455.
- Bennett, A. F. 1985. Temperature and muscle. *J. Exp. Biol.* **115**:333-344.
- Blouin-Demers, G. & Weatherhead, P. J. 2001. Thermal ecology of black rat snakes (*Elaphe obsoleta*) in a thermally challenging environment. *Ecology* **82**(11): 3025-3043.
- Brattstrom, B. H. 1965. Body temperatures of reptiles. *Am. Midl. Nat.* **73**(2): 376-422.
- Brown, G. P. & Weatherhead, P. J. 2000. Thermal ecology and sexual size dimorphism in Northern Water Snakes, *Nerodia sipedon*. *Ecol. Mono.* **70**(2): 311-330.
- Burger, J. 1998. Antipredator behaviour of hatchling snakes: effects of incubation temperature and simulated preators. *Anim. Behav.* **56**: 547-553.
- Charland, M. B. & Gregory, P. T. 1990. The influence of females reproductive status on thermoregulation in viviparous snake, *Crotalus viridis*. *Copeia* 1990: 1089-1098.
- Christian, K. A. & Tracy, C. R. 1981. The effect of the thermal environment on the ability of hatchling Galapagos Land Iguanas to avoid predation during dispersal. *Oecologia (Berl.)* **49**: 218-223.
- Claussen, D. L., Lim, R., Kurz, M., & Wren, K. 2002. Effects of slope, substrate, and temperature on the locomotion of the Ornate Box Turtle, *Terrapene ornata*. *Copeia* **2002**(2): 411-418.
- Cooper, W. E. & Vitt, L. J. 1986. Thermal dependence of tongue-flicking and comments of use of tongue-flicking as an index of squamate behaviour. *Ethology* **71**: 176-186.
- Crowley, S. R. & Pietruszka, J. 1983. Aggressiveness and vocalization in the leopard lizard (*Gamgelia wislizenii*): the influence of temperature. *Anim. Behav.* **31**: 1055-1060.
- Currin, S. & Alexander, G. J. 1999. How to make measurements in thermoregulatory studies: the heating debate continues. *African J. Herpetol.* **48**(1&2): 33-40.
- Dreisig, H. 1980. Daily activity, thermoregulation and water loss in the tiger beetle *Cicindela hybrida*. *Oecologia (Berl.)* **44**: 376-389.

- Dunham, A. W. 1978. Food availability as a proximate factor influencing individual growth rates in the iguanid lizard *Sceloporus merriami*. *Ecology* **59**: 770-778.
- Elsworth, P. G., Seebacher, F., & Franklin, C. E. 2003. Sustained swimming performance in crocodiles (*Crocodylus porosus*): Effects of body size and temperature. *J. Herp.* **37**(2): 363-368.
- Gibson, A. R. & Falls, J. B. 1979. Thermal biology of the Common Garter Snake *Thamnophis sirtalis*. I. Temporal variation, environmental effects and sex differences. *Oecologia* **43**: 79-97.
- Gibson, A. R., Smucny, D. A., & Kollar, J. 1989. The effects of feeding and ecdysis on temperature selection by young Garter Snakes in a simple thermal mosaic. *Can. J. Zool.* **67**:19-23.
- Grant, B. W. & Dunham, A. E. 1988. Thermally imposed time constraints on the activity of the desert lizard *Sceloporus merriami*. *Ecology* **69**(1): 167-176.
- Greenwald, O. E. 1971. Thermal dependence of striking and prey capture by gopher snakes. *Copeia* **1971**: 141-148.
- Gutzke, W. H. & Packard, G. C. 1987. Influence of the hydric and thermal environments on eggs and hatchlings of bull snakes *Pituophis melanoleucus*. *Physiol. Zool.* **60**(1): 9-17.
- Hailey, A. 1984. Ecology of the viperine snake, *Natrix maura*. PhD thesis, University of Nottingham.
- Hertz, P. E., Huey, R. B., & Nevo, E. 1982. Fight versus flight: Body temperature influences defensive responses of lizards. *Anim. Behav.* **30**: 676-679.
- Hertz, P. E., Huey, R. B., & Stevenson, R. D. 1993. Evaluating temperature regulation by field-active ectotherms: The fallacy of the inappropriate question. *Am. Nat.* **142**(5): 796-818.
- Hertz, P. E., Huey, R. B., & Stevenson, R. D. 1999. Temperature regulation in free-ranging ectotherms: what are the appropriate questions? *African J. Herpetol.* **48**: 41-48.
- Huey, R. B. 1982. Temperature, physiology and the ecology of reptiles. In *Biology of The Reptilia* v. 12. (Gans, C. & Pough, F. H. eds.). Academic Press, London. pp: 25-74.
- Huey, R. B. & Pianka, E. R. 1977. Seasonal variation in thermoregulatory behaviour and body temperature of diurnal Kalahari lizards. *Ecology* **58**: 1066-1075.
- Kingsbury, B. A. 1994. Thermal constraints and eurythermy in the lizard *Elgaria multicarinata*. *Herpetologica* **50**(3): 266-273.

- Lillywhite, H. B. 1987. Temperature, energetics and physiological ecology. In Snake: Ecology and Evolutionary Biology. (Siegel, R.A., Collins, J.T. & Novak, S.S. eds.). McGraw-Hill Publishing Company, Toronto. pp. 422-477.
- Manning, B. & Grigg, G. C. 1997. Basking is not of thermoregulatory significance in the "basking" freshwater turtle *Emydura signata*. *Copeia* **1997**(3): 579-584.
- Marsh, R. L. & Bennett, A. F. 1986. Thermal dependence of sprint performance of the lizard *Sceloporus occientalis*. *J. Exp. Biol.* **126**: 79-87.
- Paterson, J. W. & Davies, P. W. 1978. Preferred body temperature: seasonal and sexual differences in the lizard *Lacerta vivipara*. *J. Therm. Biol.* **3**: 39-41.
- Peterson, C. R. 1987. Daily variation in the body temperatures of free-ranging Garter Snakes. *Ecology* **68**(1): 160-169.
- Shine, R. & Madsen, T. E. 1996. Is thermoregulation unimportant for most reptiles? An example using Water Pythons (*Liasis fuscus*) in tropical Australia. *Physiol. Zool.* **69**(2): 252-269.
- Stevenson, R. D. 1985. The relative importance of behavioural and physiological adjustments controlling body temperature in terrestrial ectotherms. *Am. Nat.* **126**(3): 362-386.
- Stevenson, R. D., Peterson, C. R., & Tsuji, J. S..1985. The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the Wandering Garter Snake. *Physiol. Zool.* **58**(1): 46-57.
- Tanaka, H., Takahi, Y., & Naito, Y. 2000. Behavioural thermoregulation of Chum Salmon during homing migration in coastal waters. *J. Exp. Biol.* **203**: 1825-1833.
- Weatherhead, P. J. & Robertson, I. C. 1991. Thermal constraints on swimming performance and escape response of Northern Water Snakes, *Nerodia sipedon*. *Can. J. Zool.* **70**: 94-98.

## GENERAL CONCLUSIONS

1. The natural history of Grass Snakes at Fordwich, UK was broadly similar to populations studied elsewhere in Europe. Common features of this species' natural history include activity over a wide range of operative temperatures ( $T_{es}$ ), pronounced sexual size dimorphism and a diet that consists principally of anurans.
2. Spot temperature measurements in the field showed that, although Grass Snakes at Fordwich were active over a wide range of body temperatures ( $T_{bs}$ ) in the field (18 °C to 31.5 °C), they appeared to thermoregulate only at moderate to high  $T_{es}$  (25 °C to 30 °C).
3. Data from an experiment monitoring snake  $T_{bs}$  in an outdoor enclosure showed that thermoregulatory opportunities for Grass Snakes were limited to daytime periods (0800-2000), which is similar to studies of other snakes at high latitudes.
4. Grass Snakes in the experimental enclosure thermoregulated at high  $T_{es}$  ( $T_e = 38.4$  °C, corresponding with a  $T_b$  of 27.7 °C) and maintained fairly stable  $T_{bs}$  as  $T_{es}$  increased. These data were consistent with observations of  $T_{bs}$  in free-ranging snakes.
5. Contrary to expectation, gravid females maintained lower and more variable  $T_{bs}$  than nongravid females. By contrast, in studies of other temperate-zone snakes, gravid females have been shown to thermoregulate more precisely and at higher  $T_{bs}$  than nongravid females.
6. Snakes in a thermogradient selected relatively high  $T_{bs}$  in the laboratory (preferred  $T_{bs} = T_{set}$ ). Even though there was tremendous variability in  $T_{set}$

between individuals,  $T_{set}$  generally exceeded those values in the literature for other natricines.

7. Tests of crawling and swimming speeds of snakes in the laboratory showed these behaviours to be highly temperature-dependent. Mean and maximum speeds occurred over a broad range of high  $T_b$ s consistent with expectations for a high-latitude ectotherm. Furthermore,  $T_b$ s for maximum performance were consistent with temperatures selected in a thermogradient and with  $T_b$ s of thermoregulating snakes in the field. Tongue-flick rates, however, were relatively independent of temperature, but I cannot rule out experimental design flaws in this case.