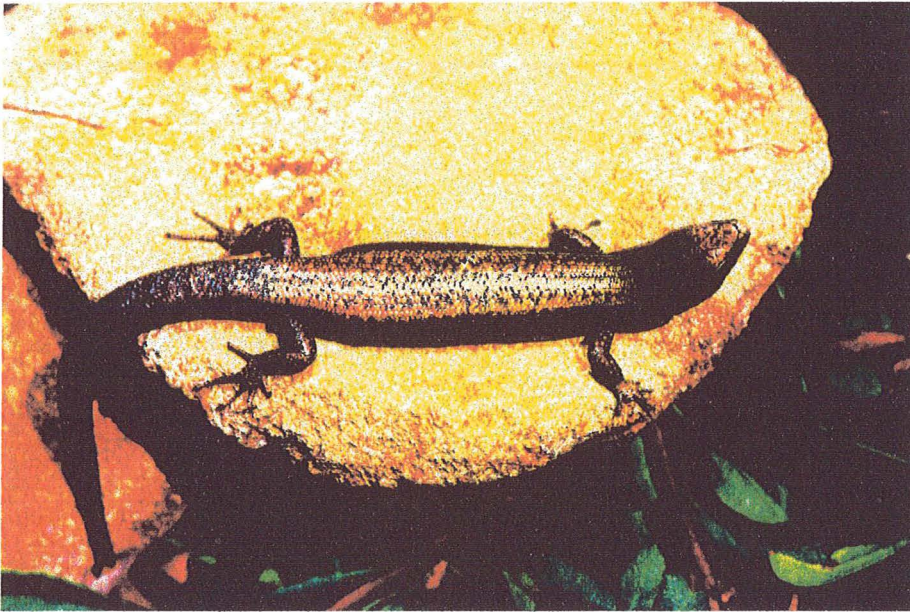

**MATERNAL COSTS OF
REPRODUCTION IN THE
SOUTHERN SNOW SKINK:**
Niveoscincus microlepidotus



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Submitted in fulfillment of the requirements for the degree of Bachelors of Science
with Honours

School of Zoology
University of Tasmania
1999

ABSTRACT

Viviparous reproduction is associated with many potential costs that may contribute to the need to adopt a number of physiological and behavioral tradeoffs. Costs are divided into two main categories, survival and fecundity, and these may manifest as locomotory, thermoregulatory, and metabolic costs. Animals must balance these costs with the benefits of reproduction to evolve a successful life-history. To increase our understanding of the life-histories of viviparous reptiles, this study investigated the changes in mobility, thermoregulatory behavior, thermal physiology, and metabolism during pregnancy in *Niveoscincus microlepidotus*.

Niveoscincus microlepidotus is a small (3 - 5g) alpine specialist, restricted to altitudes above 1080 m in Tasmania, Australia. Its preferred microhabitat is open heathlands, alpine forests, and dolerite boulder-fields. This species has an unusual reproductive cycle that makes it an ideal study species for this type of study. Ovulation and mating occur in spring to early summer, gestation takes place during summer and early autumn. The fully developed embryos are held through the winter hibernation and parturition occurs the following spring. Mating does not occur until the next spring. This biennial reproductive mode allows pregnant and non-pregnant females to be studied simultaneously.

This study has shown that female *Niveoscincus microlepidotus* decrease body temperature during pregnancy. Presumably, this is in order to slow embryonic development until just prior to the commencement of hibernation. This may have substantial advantages for reducing the costs of pregnancy to the pregnant female and any costs to the neonates.

It has also been shown that *Niveoscincus microlepidotus* incurs a significant decrease in mobility during pregnancy, which is exaggerated by high temperature and therefore increasing costs as gestation continues. However, it was also found that this species may temporally shift its physiological optimal performance temperature to minimise

the decrease in performance seen at higher temperatures. Many of the costs of reproduction associated with impaired locomotory ability are thereby decreased..

This study also examined changes in the standard metabolic rate (SMR) and active metabolic rate (AMR) associated with viviparous pregnancy. This was accomplished by measuring oxygen consumption for SMR and biological elimination of the rubidium⁸⁶ isotope for AMR. It was shown that there was a significant increase in SMR that reached a peak in late pregnancy, and AMR was significantly higher in pregnant females than non-pregnant females. This suggests that there is a significant metabolic cost associated with pregnancy in female *N. microlepidotus*. The rubidium⁸⁶ isotope was proved to be a useful method for determining active metabolic rates in the field, minimising many of the problems inherent in other radionuclide metabolic techniques.

This study has shown that *Niveoscincus microlepidotus* uses a range of physiological and behavioral adaptations to minimise the costs of reproduction. Thereby, *N. microlepidotus* has therefore evolved a successful life-history that has allowed it to thrive in its variable alpine microhabitat.

ACKNOWLEDGMENTS

I would like to thank many people here at the University of Tasmania for accepting a yank and making him feel more at home here than anywhere else in the world.

First I would like to thank Dr. Roy Swain my supervisor and Jedi master for putting up with my awful writing though the year, his advice and guidance about my project, and most of all for encouraging me to have “the potential to be quite adequate”.

Dr. Sue Jones, who help me though out the year, even thought she was not actually my co-supervisor she always made me feel welcome in her office.

Dr. Randy Rose, for the use of his laboratory, and all his help with the metabolic work, and talks about guitar gods.

The lizard hunters, Miles Lawler, Tam Kincade, Colin McCoull:

Colin: for your help in the field, the laboratory, with statistics, and with my thesis. Thanks for all the talks, being a good sounding board and your encouragement throughout the year.

Miles: the lizard hunting god, who could always help me get more lizards than needed, but most of all for taking me handfish diving.

Tam: the batty one her self. Thank you for all your help in the field, the laboratory, editing of my thesis, and for all the nights bat trapping. ☺

I would like to thank my flatmate Chloë Showell, who I know thought I was completely mad, but was always a good flatmate anyway.

Thanks to Yvonne Vucica, for your help editing my thesis.

Thank you to my parents who know I am mad, but love me any way.

Thanks to the little *Niveoscincus microlepidotus* that “volunteered” their time, and bodies, without whom this project would have never been possible.

My friends Miles and Tam: for the few days not working, without which I would not have made it through, and who have made this the best year of my life so far.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

It has been an enduring belief that procreation of the species is the “goal” of all life. It is also widely agreed that reproduction has a great many costs associated with it. Examples of these costs may be higher mortality, reduced energy stores, or decreased maternal growth (Madsen and Shine, 1993; Schwarzkopf, 1996). These costs are believed to be the fundamental constraints on the evolution of many aspects of life-history and reproduction. In order to minimize these costs, evolutionary “trade-offs” between different components of an organism’s life have been adopted. Some examples of these trade-offs are the age of sexual maturity, reproductive frequency, and lifetime fecundity (Reznick, 1992).

Two main categories of reproductive costs have been identified in reptiles: those affecting survival and those influencing fecundity (Shine, 1980). Survival costs are the conditions of current reproductive effort that determine an individual’s chance of survival. Fecundity costs are the consequences of current conditions that may affect future reproductive output. Both costs may be direct or indirect; indirect costs include such factors as reduced growth, and reproductive modality. Direct costs include reductions in energy stores, and decreased maternal fitness (Williams, 1966; Stearns, 1989). Reproduction involves both physiological costs (Birchard *et al.*, 1984; Beuchat and Vleck, 1990; DeMarco and Guillette, 1992; DeMarco, 1993), and ecological costs (Shine, 1980; Beuchat, 1986; Brodie, 1989; Schwarzkopf and Shine, 1992; Madsen and Shine, 1993) both of which may affect many aspects of a successful life-history.

An optimal life-history must evolve within appropriate constraints in order to balance the costs of reproduction with the greatest possible reproductive success. This simple

equation is the driving force behind the physiological and behavioural adaptations associated with reproduction.

Australian reptiles exhibit a wide range of reproduction patterns that, until recently, have received relatively little attention. Although much of the emphasis has been on ecological aspects of reproduction and reproductive strategies, particular questions about reproductive trade-offs have been considered in only a few species (Resnick, 1985; Doughty and Shine, 1997).

One fundamental reproductive adaptation is viviparity. It has evolved in all classes of vertebrates, with the exception of birds and cyclostomes; however, it is relatively uncommon within amphibians and teleost fishes (Shine, 1989). Among reptiles viviparity has evolved in almost 20% of the species (Blackburn, 1982). Approximately one-third of squamate reptiles have adopted a viviparous reproductive mode (Shine, 1985a). However, 71% of these reptiles are found in two lizard families (Iguanidae and Scincidae), and two families of snakes (Colubridae and Viviperidae; Shine, 1989). These viviparous reptiles have developed diverse levels of reproductive specialization, ranging from simple egg retention, in which the large yolk is assumed to provide all the nutrients necessary for embryonic development, to a complex placentotrophy comparable to the specialisation found in many eutherians (Blackburn *et al.*, 1984). The degree of placental nutrient transfer in the majority of viviparous Australian squamates is unknown, but most viviparous skinks have a simple chorio-allantoic placenta with a large yolk sac, comparable to that of similar-sized oviparous species (Weekes, 1935; Stewart and Thompson 1994). Few of these taxa contain both viviparous and oviparous forms, although this dual reproductive mode does exist in a few species of diplodactyline geckos, elapid snakes, and lygosomine skinks (Shine, 1977, 1985a; Cree and Guillette, 1995). In these species reproductive bimodality is correlated with distinct differences in climate and habitat distribution (Shine, 1985).

It has been suggested that viviparity has evolved in reptiles as a response to cold climates or environmental instability (Heatwole, 1976; Tinkle, 1977; Shine, 1983, 1985). Occupation of a cold climate is the most consistent correlate with viviparity.

However, not all cold climate reptiles are viviparous and not all viviparous lizards inhabit a cold climate. The presence of viviparous reptilian taxa that do not inhabit a cold climate may be due to evolutionary radiations subsequent to the evolution of this trait (Shine, 1989).

Ectothermic reptiles depend on external temperatures to facilitate embryonic development, and these temperatures must remain within a very specific range in order for proper development to occur. In a cold climate, retention of the embryo allows greater control of the thermal environment that the embryo is exposed to through the mother's thermoregulatory behavior. Oviparity in these situations may lead to the eggs being laid in situations in which temperatures may be below optimal, causing development to proceed too slowly (McCoy, 1968; Guillette, 1993). Oviparous species require relatively long, stable, and warm seasons to complete egg incubation. The ability of viviparous reptiles to control their body temperature through thermoregulatory behaviour is thought to permit embryos to be maintained at an optimum temperature for development. This is in contrast to the environmental fluctuations experienced by the developing embryos of oviparous species (McCoy, 1968).

Reproductive mode is only one aspect of a successful life-history. Many reptiles have adapted their reproductive cycle to lessen the associated costs. Saint-Girons and Kramer (1963) described two major types of reproductive cycles for female lizards: annual and biennial. Annual reproduction occurs when the female produces offspring on a yearly basis. Biennial reproduction is where the female delays reproduction for a year after giving birth to the current offspring. The biennial cycle is often characteristic of cold climate reptiles (Heatwole 1976; Cree and Guillette, 1995). Biennial cycles often result from a reduced activity season which does not provide sufficient foraging time for a female to accumulate the energy necessary for egg formation, maternal growth, and the production of adequate fat stores for hibernation (Heatwole 1976; Cree and Guillette, 1995). Because there is insufficient time to obtain enough food to both maintain a normal metabolism and accumulate the

reserves necessary for egg formation, a second season is often required before reproduction can occur again.

In most biennial species, the male cycle is unrelated to the female cycle and are therefore able to mate annually. The females of some species are able to store ova or sperm after mating for long periods and consequently the mating and fertilisation of eggs may be considerably separated in time (Smyth and Smith, 1968; Heatwole, 1976).

1.2 LIZARD FAUNA OF AUSTRALIA

The Australian lizard fauna consists of more than 450 species from five families: Agamidae, Gekkonidae, Pygopodidae, Scincidae, and Varanidae. The Scincidae is the largest family, with over 270 species described in Australia (Cogger, 1992) and comprises almost 57% of all known lizards (Wilson and Knowles, 1988). Skinks are found throughout Australia from lowland to alpine regions (Greer, 1989). The Tasmanian reptilian fauna, in contrast, has been described, by Heatwole (1976) as relatively impoverished, embodying only three reptile families. It contains only 1 species of agamid, 17 species of skinks and 3 species of elapid snakes (Heatwole, 1976). Seventeen of these species are viviparous (fourteen skinks and all three snakes). The majority of the Tasmanian skinks were once classified within the genus *Leiopisma*. Recently Hutchinson *et al.* (1990) revised this genus in a major taxonomic and genetic study, resulting in the creation of a number of new genera. One of the newly erected genera is *Niveoscincus*, or the “snow skink”. This genus is comprised of 8 species: *Niveoscincus microlepidotus*, *N. greeni*, *N. ocellatus*, *N. orocryptus*, *N. palfreymani*, *N. pretiosus*, *N. metallicus*, and *N. coventryi*. Little published research is available for the ecology of these skinks. Melville and Swain (1998; 1999) have provided a phylogeny for the genus based on the cytochrome *b* gene, and Melville (1998) conducted an extensive eco-morphological study of the eight species.

1.3 STUDY SPECIES

The genus *Niveoscincus* comprises a group of small to medium-sized lygosome lizards, characterised by the possession of a well-developed prefrontal shield, paired or fused frontoparietals, a distinct interparietal shield, and a moveable lower eyelid with a moderate to large transparent palpebral disc. All species in this genus have well-developed pentadactyl limbs (Hutchinson *et al.*, 1989).

Niveoscincus is the largest genus found in Tasmania, and 6 of the 8 described species are endemic. *Niveoscincus metallicus* is found both on the mainland of Australia and in Tasmania, and *Niveoscincus coventryi* is the only species found exclusively on the mainland. *N. microlepidotus*, *N. orocryptus*, and *N. greeni* are the only true alpine species found in Tasmania. *Niveoscincus microlepidotus* will be the focus of this study and is widely distributed throughout Tasmania in the alpine regions of Mount Wellington, the Cradle Mountain Range, Mount Field, and the Hartz Mountain Range (Table 1.1 and Figure 1.1).

Table 1.1 Locations of *Niveoscincus microlepidotus* across Tasmania, with identifier on Figure 1.1

Location	Maximum height of range m ASL	Number on Figure 1.1
Mount Wellington	1270m	1
Collins Bonnet	1140m	2
Hartz Mountain	1255m	3
Picton Mountain	1327m	4
Precipitous Bluff	1120m	5
Mount Field	1434m	6
Mount Bowes	1240m	7
Prince of Wales Range	1128m	8
Frenchmans Cap	1443m	9
Mount Sorrell	1144m	10
Mount Remus	1110m	11
Pyramid Mountain	1130m	12
Cradle Mountain Range	1545m	13

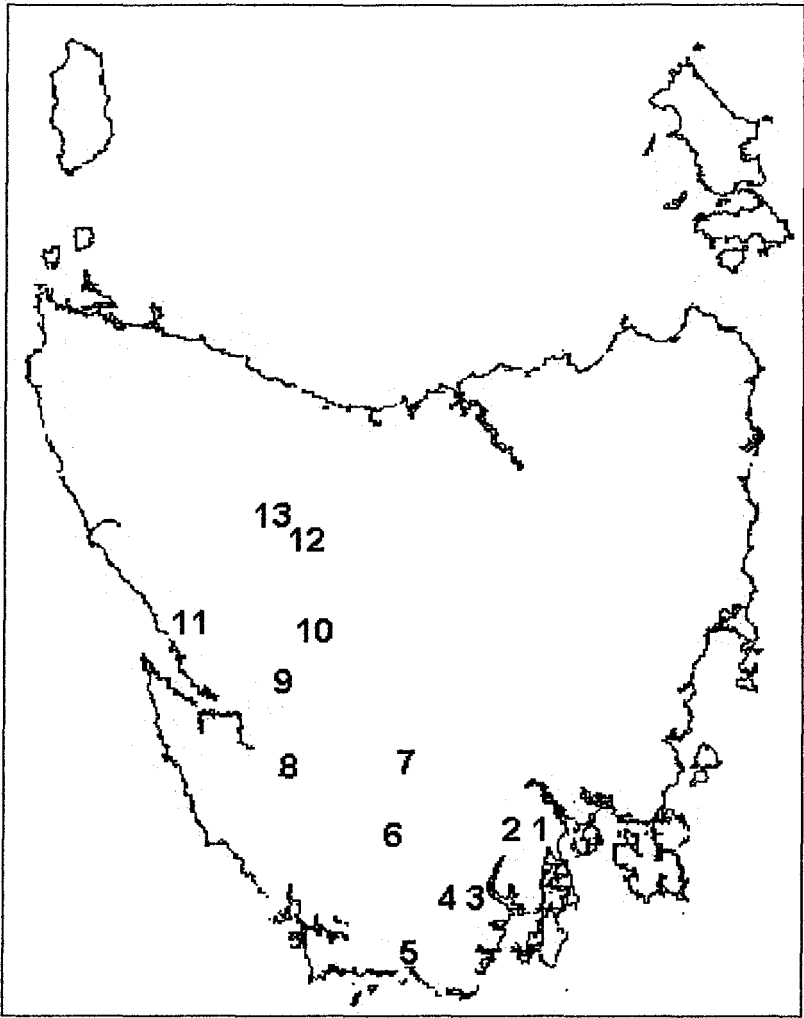


Figure 1.1 Map of Tasmania showing the known locations of *Niveoscincus microlepidotus* as described by Taylor (1991) for Forestry Commission Tasmania; codes are identified in Table 1.1

Niveoscincus microlepidotus is an alpine species restricted to mountain-tops above 1080 m asl in the southern highlands of Tasmania, including Mt. Wellington (Hutchinson and Schwaner, 1991). It is a heath-dwelling species that is found in open heathlands and dolerite boulder fields that characterise its alpine habitat (Melville and Swain 1997a). *N. microlepidotus* is one of the larger skinks in the *Niveoscincus* genus. Adults weigh between 3 - 5 g, have a snout-vent length of 50 - 69 mm, and can reach a total length of 170 mm. Mid-body scales are in 32 - 44 rows (“microlepidotus” means “small scales”), which are often used as a species identifier. Colouration of the

dorsal head, body, and tail is greyish to olive with scales that are flecked and edged with black, forming a crude checker-board pattern. The dorsolateral scale row is weakly flecked or unmarked, producing a light stripe. The upper lateral zone is dark-grey to black, and the lower lateral zone is greyish and black-flecked. The ventral surface is a lighter grey (Hutchinson *et al.*, 1989).

Niveoscincus microlepidotus is a sexually dimorphic lizard. The male has a larger more pronounced head than that of the female. The female is characterized by smaller body size and has a less pronounced neck (Figure 1.2; Melville, 1994).

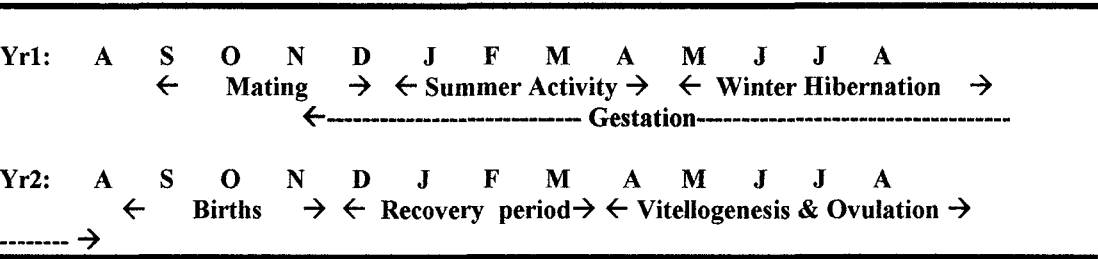


Figure 1.2 *Niveoscincus microlepidotus*; pregnant female on left and non-pregnant female on right

Niveoscincus microlepidotus has one of the most unusual reproductive cycles known in lizards (Olsson & Shine, 1998). Mating occurs in late spring to early summer (October to December). The mother carries 1 - 4 embryos, which, although fully

mature and viable by the end of autumn (April), are retained within the uterus through the winter during hibernation and are born the following spring (September through November; Figure 1.3). In non-alpine *Niveoscincus* species, parturition occurs during the summer or early autumn, giving the newborn animals ample time to prepare for the winter (Swain & Jones 1994). By delaying parturition until after winter *N. microlepidotus* may enhance their offsprings' chance of survival (Olssen and Shine 1998). Neonates born in the spring will face better climatic conditions and higher food availability. This unusual gestation cycle effectively makes a biennial reproductive strategy obligatory.

Figure 1.2 Biennial reproductive cycle of female *Niveoscincus microlepidotus*



Niveoscincus microlepidotus was chosen for this study because of the availability of a large population on Mt. Wellington (near Hobart in southern Tasmania) and the reproductive characteristics of this species. Because this species reproduces biennially, pregnant and non-pregnant females co-occur, providing an opportunity to examine costs associated with pregnancy by direct comparison.

1.4 RESEARCH OBJECTIVES

The primary aim of this research thesis is to describe physiological and behavioural responses associated with pregnancy in the Southern Snow Skink, *Niveoscincus microlepidotus*, using both laboratory and field observations. This major aim was addressed by investigating the following series of related questions.

- Does pregnancy affect locomotory behaviour?
- Is pregnancy associated with changes in body temperature?
- Is pregnancy associated with changes in metabolic rate?

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 STUDY SITE

The island state of Tasmania is found approximately 200 km from the southern tip of the Australian mainland. It is located between latitudes 40- 45 °S and longitudes 140- 150 °E. Tasmania is composed of mountainous and lowland areas, which have a cool to cold temperate maritime climate (Figure 2.1).

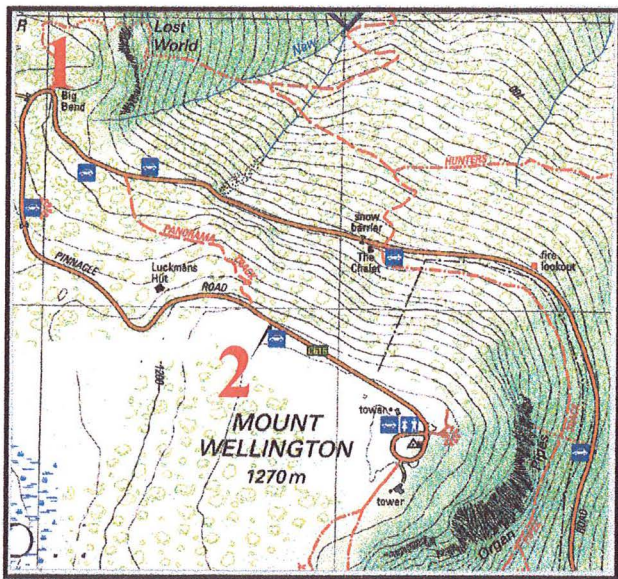


Figure 2.1 Map of Tasmania indicating location of field site. General study areas at Mount Wellington are identified: 1) lower study site; 2) and upper site.

The study site chosen for this research was located near the summit of Mount Wellington (147° 13' E 42° 53' S). The mountain is located on the edge of the Derwent River Valley, and is approximately 8 km from the city of Hobart in southeastern Tasmania. Two study sites were used; site 1 was located at 1120 m a.s.l. near a popular walking track (The Big Bend track); site 2 was located at 1240 m a.s.l. which is rarely disturbed by humans (Figure 2.1). Three distinct microhabitats were

identified alpine heathfields (Figure 2.2a), alpine woodlands, and dolerite boulder fields (Figure 2.2b). These microhabitats occur, in small patches with distinct boundaries at these sites

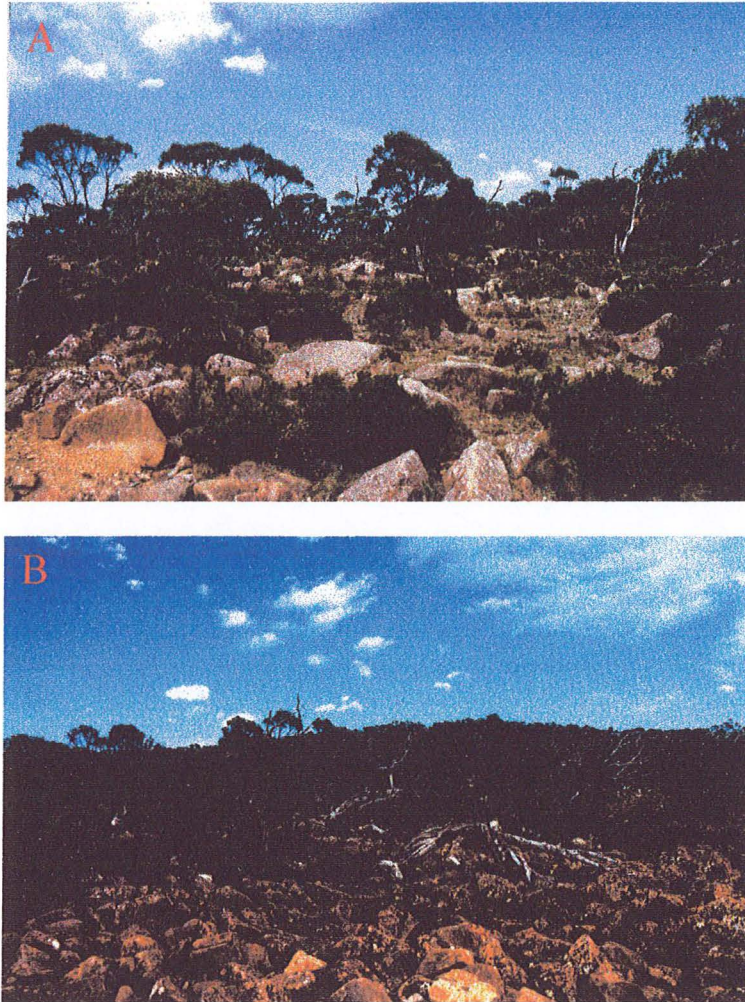


Figure 2.2 Field site at Mount Wellington: a) Mixed heath and boulder habitat and b) dolerite boulder field (at 1240 m asl).

The ragged, rocky mountains of Tasmania have been shaped by erosion prior to and during the Pleistocene glacial period. The majority of the glacial erosion occurred on the eastern lee sides of these mountains. Periglacial and glacial action formed the relatively common large moraine-dolerite boulder fields, where vegetation growth is limited (Department of Environment and Land Management, 1997).

The alpine zone occurs in Tasmania at approximately 1000 m a.s.l (Kirkpatrick, 1997). The climate within this zone is cool to cold throughout the year. No months are frost-free, and snow and glazing storms may occur at any time during the year. This, combined with the poor soil caused by periglacial soilifuction activity, affects the height and shape of most of the vegetation (Odgen and Powell, 1979). The soil types range from a deep clay base to thin acidic soils, and in the wetter areas there is a high organic content tending towards peat. The study sites contain many endemic and unusual species of flora (Kirkpatrick., 1997; Table 2.1). *Eucalyptus coccifera* dominates the majority of the canopy, at site 1 reaching a maximum height of about 10 m and allowing light to penetrate easily. This contrasts to site 2 in which trees are scarce and the habitat is very exposed and open.

Table 2.1* Flora found in study sites located on Mt Wellington

Plant Family	Plant Species
Epacridaceae	<i>Cyathodes parvifolia</i>
	<i>Dracophylla minimum</i>
	<i>Donatia novaezelandiae</i>
	<i>Epacris serpyllifolia</i>
	<i>Richea scoparia</i>
Compositiae	<i>Helichrysum hookeri</i>
Gleicheniaceae	<i>Gleichenia alpina</i>
Liliaceae	<i>Astelia alpina</i>
Myrtaceae	<i>Leptospermum lernigerum</i>
Proteaceae	<i>Banksia marginata</i>

*from Kirkpatrick, 1997

At site 1, the skink fauna is limited to three species: *Niveoscincus microlepidotus*, *N. ocellatus* and *N metallicus*. However, at site 2 (approx. 1240 m asl) *N. microlepidotus* is the only lizard present and occurs in very large numbers. It has been estimated that there may be as many as 1000 *N. microlepidotus* per hectare present (Swain per comm.). There have also been reports of *Notechis ater* (black tiger snake), *Drysdalia coronoides* (whip snake) and *Cyclodomorphus casuarinae* (she-oak skink) found at both sites (Melville 1994).

2.2 LIZARD COLLECTION AND HANDLING

Three methods were used to collect animals from the study sites. The first used a “lizard noosegun”, which is a modified fishing rod with a fishing line noose attached to the end. The noose was positioned over a lizard’s head and tightened quickly to catch the animal. The second method involved baiting animals with a mealworm (*Tenebrio* larva) attached to a fishing line and rod. The lizards would bite the worm and clamp down, remaining attached until grabbed by hand or shaken into a bucket. However, these methods limited the capture of basking or foraging lizards. During inclement weather animals were caught by hand by turning over rocks, exposing the lizards. However, this method would only allow the collection of cold, immobilized lizards.

Female lizards were collected in batches of 20 - 30 animals. *Niveoscincus microlepidotus* is abundant at both sites and removal of this number of animals had no observable impact on the abundance or the ecology of this species.

Animals were transported back to the laboratory in calico bags with a small amount of leaf litter. Once in the laboratory, snout-vent length (SVL) was measured using vernier calipers to ± 0.1 mm. The animal were weighed using a Sartorius[®] digital scale to ± 0.01 g, sex was determined by eversion of the hemipenes if present, and reproductive status of females was determined by palpation.

2.3 LABORATORY HOUSING

The lizards were kept in the University of Tasmania lizard housing facility. Animals were provided with light by fluorescent tubes on a 14-hour light/dark cycle, providing an intensity of approximately 20,000 lux. The ambient room temperature was maintained at approximately 12 °C by an air-conditioner. In the laboratory, animals were housed in a plastic containers (20 X 30 X 10 cm) with mesh tops, and absorbent bedding. A 25 watt basking light was suspended above one end of each container,

providing a temperature gradient of 12-35 °C. The basking lamps were controlled by automatic timers and were activated for 9 hours per day. Uprturned terra-cotta bowls were provided for shelter and basking sites. Food and water were provided *ad libitum*. The animals were fed three times weekly on a diet on mealworms (*Tenebrio larva*), cat food, and mashed banana.

2.4 ENCLOSURE HOUSING

Lizards used for the first rubidium elimination experiments were housed in outdoor enclosures to best simulate field conditions. These enclosures were in the animal care facility at the University of Tasmania. The circular enclosures consisted of galvanized sheet metal with a diameter of 1.5 m and a height of 50 cm. The base of the enclosure was set into the ground approximately 15 cm on a level base of gravel and sand for drainage. The bottoms of the enclosures were sealed with flyscreen mesh to prevent escape. The enclosure tops were covered with a fine nylon fish netting and chicken wire to prevent escape and predation. Bark and rocks were placed in each enclosure for shelter and basking. Water was supplied *ad libitum* and, although there was some natural food available, the animals were fed three times per week on the same diet as the animals housed in the laboratory.

Four animals were kept in each enclosure, two gravid and two non-gravid females. They were marked with a non-toxic white marking pen for identification. However, the marker faded rather quickly so the animals were remarked every time they were removed for measurement of radioactivity.

2.5 PERMITS

The research described here was carried out under Ethics Permit 98076 issued by the University of Tasmania Animal Ethics Committee to Associate Professor Roy Swain.

All collection and field observations were performed by permission of the Hobart City Council permit number 2/98 (by-law 148) granted to Alexander Kabat.

CHAPTER 3

THERMOREGULATORY CHARACTERISTICS

3.1 INTRODUCTION

Reptiles maintain body temperatures that reflect their current physiological status. Body temperatures are controlled behaviourally and may vary depending on time of day, season, digestive status, and reproductive status (Harwood, 1979; Huey, 1982; Beuchat, 1986; Andrews and Kenney, 1990; Werner, 1990). Temperature strongly influences a number of behavioural and physiological processes in ectothermic animals, including predator avoidance (Christian and Tracy, 1981; Hertz *et al.*, 1982; Greene, 1988; Losos, 1988), mobility (Avery and Bond, 1989; Brodie, 1989; Van Damme *et al.*, 1989), and reproduction (Pilorge and Barbault, 1981; Duvall *et al.*, 1982; Beuchat and Ellner, 1987). Consequently, temperature is the single most important physical factor in the ecology of a reptile (Heatwole, 1976; Blazquez, 1995). Thermoregulation is achieved through modification of activity times, basking behaviours, and use of microhabitat.

An ectotherm's physiological processes and the intraspecific variation of these, may require the selection of different "optimum" body temperatures to maximise physiological functions (Huey, 1982; Christian and Tracy, 1983). Consequently, many ectotherms regulate their body temperatures (the ecritic temperature) between upper and lower thermal setpoints by moving between different thermal microclimates (Patterson and Davies, 1978; Huey, 1982; Van Berkum *et al.*, 1986). These upper and lower setpoints are believed to be controlled by hypothalamic temperature receptors that determine the temperatures at which behavioural thermoregulation (basking) begins or ceases (Jones and Avery, 1989; Tosini and Avery, 1993). Ambient temperature, body size, sexual status, risk of predation and a lizard's ability to regulate heat exchange with the environment also influence time spent thermoregulating (Carrascal *et al.*, 1992). *Niveoscincus microlepidotus* controls

body temperature by both heliothermic basking and by thigmothermy (Melville and Swain, 1997b).

Efficient thermoregulation is an essential consideration for viviparous lizards. This is because maternal body temperature is known to affect the gestation length, sex ratio, viability, and size of the offspring (Avery, 1982; Beuchat, 1988; Schwarzkopf and Shine, 1991; Daut and Andrews, 1993). Usually only a very limited range of incubation temperatures allows proper development of the foetus (Shine, 1980; Tosini and Avery, 1996b; Mathies and Andrews, 1997). Therefore, pregnant female viviparous reptiles often maintain body temperatures during the reproductive periods that differ from those maintained outside of gestation (Shine, 1980; Tosini and Avery, 1996b; Mathies and Andrews, 1997). This results in pregnant females maintaining body temperatures that are suitable for embryonic development but are as close as possible to the optimal temperature for their own physiological functions (Garrick, 1974; Stewart, 1984; Beuchat and Ellner, 1987). Consequently, a pregnant female may need to maintain a body temperature that is a compromise between her own thermal optima and the optimal for embryonic developmental (Beuchat and Ellner, 1987; Schwarzkopf *et al.*, 1992). This compromise is critical, since if the temperature is too high or too low it can result in reduced viability or increased abnormalities of the young (Garrick, 1974; Stewart, 1984; Beuchat and Ellner, 1987). Pregnant lizards may thus be required to alter the frequency and duration of their basking. Such changes may affect the time available for other necessary activities.

Thermal environments are strongly influenced by altitude. Many high altitude lizards show variation in their thermal physiology and thermoregulatory behaviour associated with these thermal environments (Hertz and Huey, 1982). *Niveoscincus microlepidotus* exhibits a number of thermoregulatory adaptations that are associated with its alpine habitat (Melville, 1994; Melville and Swain, 1997; Kik, 1998). However, little is known about the variations in body temperatures associated with pregnancy in this species. In order to further the understanding of costs and trade-offs involved in pregnancy, a laboratory and field study of thermoregulatory behaviour in

N. microlepidotus was undertaken. Consequently, in this chapter the following questions were asked.

- Is there a shift in the eccritic temperature of pregnant *N. microlepidotus*?
- Is there a shift in thermal setpoints during pregnancy?
- What characterises the thermoregulatory behaviour during pregnancy?
- What is the preferred temperature during pregnancy?
- Is there a difference in heating rates during pregnancy?

3.2 MATERIALS AND METHODS

3.2.1 COLLECTION AND PREPARATION OF ANIMALS

Males, and pregnant and non-pregnant female lizards were collected from the Mount Wellington field sites as described in Section 2.1. Lizards were not used for more than one experiment, and were housed as described in Section 2.3.

3.2.2 ECCRITIC AND THERMOREGULATORY BEHAVIOUR

This experiment investigated the thermoregulatory behaviour of pregnant and non-pregnant females. The mean eccritic temperatures, time spent basking, and the control of body temperature was examined.

A square, open-topped wooden terrarium (125 x 125 x 30 cm) was used as temporary test housing. It was divided into eight sections of identical size (56 x 30 cm), each of which was identified by a number (1–8). Each section contained sand substrate as bedding and an upturned terracotta bowl was placed at one end of each test area as a basking site. A basking heat source (25 watt light bulb) was fixed about 6 cm above the basking site and provided a maximum basking temperature of approximately 35 °C. The experiment was carried out in the lizard housing facility under normal

ambient conditions (background temperature ~15 °C). Animals were not fed 24 hours prior to a trial or during a trial, but water was available *ad libitum*.

Body temperatures were recorded at 2-minute intervals, using a program created for a previous study. A small temperature probe was inserted into the cloaca of each animal and held securely in place with strips of adhesive tape. Each probe had a lead of about 1 m to allow free movement throughout the experimental enclosure. Probes were connected to a data logger that was linked to a MacintoshTM computer. Observations indicated that the temperature probes leads did not substantially hinder the movements of lizards around their test compartments.

Prior to each trial snout-vent length was measured and reproductive status was determined by palpation for each lizard. Lizards were marked on their back with a non-toxic white marker for identification. They were then transferred into the test compartments eighteen hours prior to the commencement of the trial to allow them to become acclimated to their new surroundings. Sixty minutes prior to the activation of the heat source, the temperature probes were inserted and taped into place. Body temperature was recorded for nine hours per trial as shown in (Figure 3.1).

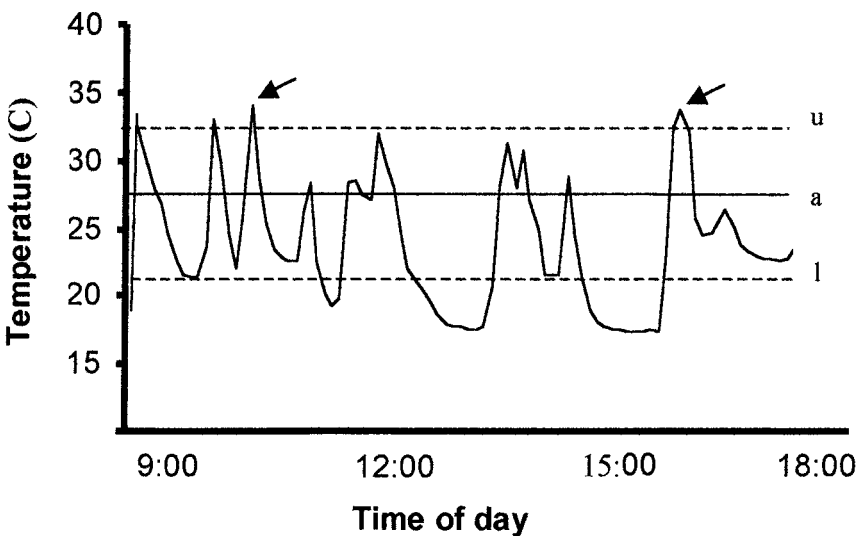


Figure 3.1 Representative body temperature data recorded for animal ‘non-pregnant 8’. Lines identify the setpoints for this animal (‘a’, ‘u’ and ‘l’ identify mean, upper and lower points respectively). The arrows identify the maximum temperature recorded for this animal.

Approximately 25 randomly selected pregnant and non-pregnant females were used for each collection period during 4 reproductive states: non-, early-, mid-, and late-pregnant. For each animal overall mean temperature, mean upper and lower thermal setpoints, maximum body temperature, frequency that body temperature reached near to a maximum, and the number of occasions when temperatures fell below 20 °C, were noted.

3.2.3 PREFERRED TEMPERATURES

Preferred temperature is the temperature that a lizard maintains when presented with a gradient, and no other influences (Dawson, 1975). This experiment investigated the preferred temperatures of pregnant and non-pregnant *Niveoscincus microlepidotus*. An aluminium box (150 x 35 x 35 cm) was used as the experimental chamber. One end of the chamber was equipped with a cooling system through which water was pumped after being cooled in an ice-bath. Three heat lamps (25, 40 and 100 watt bulbs) were positioned below the floor of the chamber approximately 30 cm apart providing increasing levels of heat to the away from the cooled area. This created a temperature gradient of approximately 5.0 °C to 45 °C. A sand substrate and water were provided, however no food, shelter or basking opportunities were supplied (Figure 3.2). This experiment was performed in the lizard housing facility under standard conditions as described in 2.3.

Body temperatures were recorded as described in Section 3.2.1 using small temperature probes inserted into the cloaca and held securely in place with strips of adhesive tape. Observations indicated that the leads did not substantially hinder the movement of the lizard throughout the testing chamber. *Niveoscincus microlepidotus* tend to be aggressive towards each other. Therefore to eliminate any intra-animal influences only one lizard was tested per trial.

Snout-vent length was measured and reproductive status was determined by palpation for each lizard. Female lizards were placed into one of two reproductive categories: pregnant or non-pregnant. Animals were placed in the box 90 minutes before the commencement of the test, and the probe was inserted 30 minutes prior to recording the first reading. Temperatures were recorded for approximately 4 hours from 10:00 h. to 14:00 h. The temperature that the animal selected for the majority of time was considered the preferred temperature for that lizard.

3.2.4 HEATING RATES

This experiment examined the differences in heating rates between pregnant and non-pregnant females. In order to do this, approximately 30 randomly selected pregnant and non-pregnant females were tested during four states of reproduction; non-, early-, mid-, and late-pregnant. A hand-held thermocouple was inserted into the cloaca and secured in place with tape. Initially each lizard was held in a plastic container placed in an ice-water bath at approximately 5 °C (± 1 °C) for 20 minutes, and was continuously monitored. Melville (1994) reported that the critical minimum temperature of *N. microlepidotus* is 2.78 °C. Therefore the 5 °C temperature imposed on the lizard caused no undue stress. Once the animal was sufficiently cooled it was transferred to another container placed in a 25 °C (± 1 °C) water bath controlled by a Ratek Instruments™ TH1 Thermoregulator. This temperature was near to the reported optimal performance temperature of 26 °C of *N. microlepidotus* (Melville 1994). Body temperature was recorded every 60 seconds until it reached 25 °C (± 1 °C) and had been maintained for at least five minutes. The resulting data were plotted against time, and the heating rate was recorded as the slope of the line of best fit.

3.2.5 FIELD TEMPERATURES

Body temperatures of 95 basking pregnant (n= 30), non-pregnant (n= 27), and male (n= 38) lizards were collected in the field for evaluation of laboratory findings. All

fieldwork was done at the upper Mt Wellington field site (site 2; see Chapter 2). Animals were collected by mealworm fishing or noosing (as described in Chapter 2). A hand-held digital thermometer was inserted into the cloaca and body temperature was recorded within 20 seconds of capture. Each animal was sexed, measured (SVL), and palpated to determine reproductive status if female. Female lizards were placed into one of two reproductive categories: pregnant and non-pregnant. Temperatures were compared to determine if there was any difference in body temperature in the field between males and pregnant and non-pregnant females. Observations on air and surface temperatures were also collected in order to assess basking site selection.

3.2.6 DATA ANALYSIS

Analyses were performed on the SAS System for Windows[®] v6.12. Homogeneity of variances was tested by visual inspection of plots of group standard deviations versus group means. Normality of data distribution was addressed by visual inspection of the log-normal plot of residuals and the plot of estimated versus predicted residual values.

For each thermal experiment the following sequence of analyses was conducted. A regression analysis was used to test the null hypothesis that size (SVL) has an effect on thermoregulatory characteristics. Rejection of this hypothesis allowed the analysis of data without the need to use SVL as a co-variate.

The data from non-pregnant animals were regressed against season to test the null hypothesis that there is a seasonal effect on thermoregulatory characteristics. The rejection of this hypothesis allowed the data obtained from non-pregnant females to be pooled in order to compare between reproductive stages (early-, mid-, and late-pregnant). Pregnant females were found at different stages during any one collection period, and were grouped by stage and not season. It was, therefore, not necessary to evaluate for any seasonal effects (see Section 3.3).

One-way ANOVAs were conducted used to test the null hypothesis that reproductive status has no effect on thermoregulatory characteristics. Where significant correlations were found, corresponding Fisher's LSD *post hoc* tests were used to identify significant groups within treatments.

3.3 RESULTS

3.3.1 LABORATORY ECCRITIC TEMPERATURES

A total of 70 pregnant and non-pregnant females was used in this experiment. A preliminary regression analysis determined that SVL had no significant effect on eccritic temperature (Table 3.1).

Table 3.1 Mean SVL and regression values of SVL against eccritic temperature of *Niveoscincus microlepidotus* during four reproductive states

State	n	mean SVL	s.e.	ANOVA		
				df	<i>F</i>	<i>P</i>
early-pregnant	13	57.7	2.94	1, 11	2.381	0.1472
middle-pregnant	11	58.9	3.06	1, 9	3.283	0.0922
late- pregnant	14	58.6	2.74	1, 12	2.198	0.6564
non- pregnant	32	59.5	2.48	1, 30	2.971	0.1296

A regression analysis determined that there was no significant seasonal effect on the body temperature of non-pregnant females (ANOVA: $F_{1, 68} = 0.361$; $P = 0.8615$). Therefore all eccritic temperature data obtained from the pregnant animals was compared to the average eccritic temperature (Table 3.2) of the non-pregnant females regardless of season .

Table 3.2 Summary of average body temperature T_b , maximum body temperature (T_{max}) and minimum body temperature (T_{min}) data in *Niveoscincus microlepidotus*, during four reproductive states

State	n	T_b (°C)	T_{max} (°C)	T_{min} (°C)
non-pregnant	32	26.9 ± 1.88	35.4 ± 1.91	16.6 ± 1.93
early pregnant	13	23.6 ± 1.59	30.6 ± 1.73	16.4 ± 2.96
mid-pregnant	11	24.1 ± 1.34	29.7 ± 2.48	16.3 ± 3.32
late-pregnant	14	23.9 ± 1.68	30.1 ± 1.87	16.5 ± 1.84

A one-way ANOVA showed that there was a significant difference in ecritic temperature (T_b) between the experimental groups (ANOVA: $F_{3, 66} = 41.38$; $P = 0.0001$). A *post hoc* Fisher's LSD test demonstrated that the non-pregnant group was significantly different from all the other reproductive states; however, there was no significant difference between any of the individual stages of pregnancy (Table 3.2).

A one-way ANOVA showed a significant decrease in the thermal maxima (T_{max}) reached by the pregnant females (ANOVA: $F_{3, 66} = 200.24$; $P = 0.0001$). A *post hoc* Fisher's LSD test showed that the significance lay between the non-pregnant group and the three reproductive stages ($P < 0.005$); and there was no significant difference between the other reproductive stages.

This shift is not mirrored by the thermal minima for pregnant females. A one-way ANOVA showed that there was no significant difference between any of the experimental groups ($P > 0.1$). This implies that *Niveoscincus microlepidotus* shifts the upper thermal setpoint during gestation to facilitate some aspect of embryonic development, thereby suggesting that higher temperatures may have a significant adverse effect on gestation rate and/or foetus health (Table 3.2 and Figure 3.3).

A one-way ANOVA showed that there was a significant difference in upper setpoint temperature (T_{move}) between the experimental groups (ANOVA: $F_{3, 66} = 39.81$; $P =$

0.0001). A *post hoc* Fisher's LSD test demonstrated that the non-pregnant group was significantly different from all the other reproductive states; however, it was shown that there is no significant difference between any of the individual stages of pregnancy (Table 3.4 and Figure 3.3). As seen in minimal temperature there was no significant difference in lower thermal setpoint (T_{bask} ; $P > 0.5$).

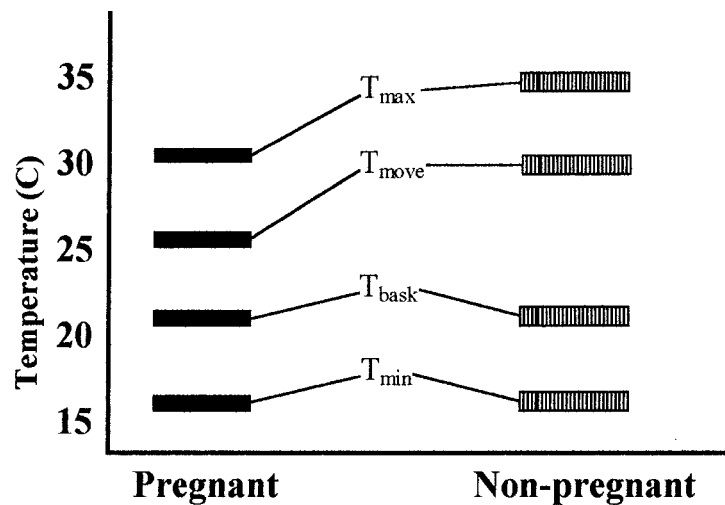


Figure 3.3 The mean thermal maxima (T_{max}) and minima (T_{min}), and mean maximal (T_{move}) and minima (T_{bask}) setpoints for the combined data from all stages of pregnant females and non-pregnant females.

Table 3.3 Summary of upper setpoint temperature (T_{move}) and lower setpoint temperature (T_{bask}) in pregnant and non-pregnant females of *N. microlepidotus*

State	n	T_{max} (°C)	T_{min} (°C)
non-pregnant	32	28.4 ± 1.12	22.1 ± 1.43
early pregnant	13	24.6 ± 2.48	21.8 ± 1.64
mid-pregnant	11	23.4 ± 2.38	22.2 ± 2.31
late-pregnant	14	24.3 ± 1.67	21.9 ± 1.26

3.3.2 PREFERRED TEMPERATURES

37 female *Niveoscincus microlepidotus* were used for this experiment. All animals were collected at the same time and were assessed as pregnant or non-pregnant; stage of pregnancy was not taken into account. Regression analyses determined that SVL had no significant relationship with preferred temperature (Table 3.4).

Table 3.4 Mean SVL and regression values of SVL against preferred temperature of female *Niveoscincus microlepidotus* during four reproductive states

State	n	mean	s.e.	ANOVA		
		SVL		df	<i>F</i>	<i>P</i>
pregnant	21	58.6	2.78	1, 19	3.991	0.4723
non-pregnant	16	57.9	3.28	1, 14	2.817	0.1232

A significant difference was shown between the preferred temperatures (T_{Pref}) of the pregnant and non-pregnant female *N. microlepidotus* (ANOVA: $F_{1, 35} = 102.10$; $P = 0.0001$). The preferred temperature of the pregnant females ($22.9\text{ }^{\circ}\text{C} \pm 1.03$) was significantly lower than that of the non-pregnant females ($26.9\text{ }^{\circ}\text{C} \pm 1.38$; Figure 3.4). This supports the conclusion drawn from the eccritic temperature data that this species lowers its body temperature during pregnancy in order to maximise embryonic development.

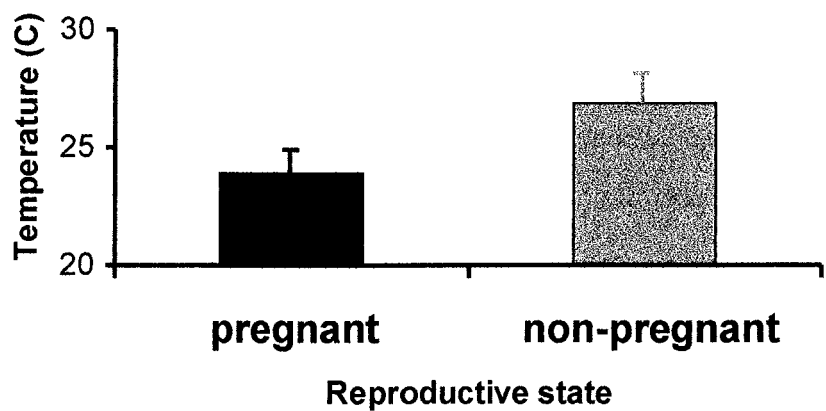


Figure 3.4 Mean preferred temperature (T_{pref} : °C) of pregnant and non-pregnant female *N. microlepidotus*

3.3.3 HEATING RATES

A total of 73 pregnant and non-pregnant females was used in this experiment. A preliminary regression analysis determined that SVL had no significant effect on heating rate (Table 3.5.)

Table 3.5 Mean SVL and regression values of SVL against heating rate of female *N. microlepidotus* during four reproductive states.

Stage	n	mean SVL	s.e.	ANOVA df	F	P
early- pregnant	11	55.7	2.91	1, 9	3.321	0.1674
middle- pregnant	13	55.9	2.62	1, 11	1.141	0.8542
late- pregnant	12	56.6	2.74	1, 10	2.918	0.5513
non- pregnant	37	57.2	2.31	1, 35	0.041	0.8396

Regression analysis of heating rates determined that there was no significant correlation between heating rate and season in the non-pregnant females (ANOVA: $F_{1, 71} = 0.453$; $P = 0.867$). Therefore heating rate data obtained from each of the

pregnancy stages were combined for comparison with the average heating rate of the non-pregnant females, regardless of season.

A one-way ANOVA showed that there was a significant difference between the heating rates of experimental groups (ANOVA: $F_{3, 69} = 16.64$; $P = 0.0001$). A *post hoc* Fisher's LSD test demonstrated that the significance was found between the late-pregnant and non-pregnant females ($P < 0.05$). However there was no significant difference between any of the other reproductive states. The average heating rate of late-pregnant females was approximately 13% slower than the heating rates of non-pregnant females (Table 3.6). This decrease in heating rate may have a substantial impact on many aspects of behaviour in this species.

Table 3.6 Mean heating rates of female *N. microlepidotus* during four reproductive states

State	n	Heating rate °C*min ⁻¹	s.e.	%diff. from non-pregnant
early-pregnant	11	2.22	0.04	3%
middle-pregnant	13	2.11	0.06	5%
late-pregnant	12	1.94	0.02	13%
non-pregnant	37	2.23	0.03	----

3.3.4 FIELD TEMPERATURES

A total of 95 animals was used for this section of this study. Animals were grouped as pregnant females, non-pregnant females, and males (Table 3.7).

Table 3.7 Mean SVL and mean body temperatures of pregnant female, non-pregnant female, and male *N. microlepidotus* in the field

Stage	n	mean SVL	s.e.	Field temperatures	
				°C	s.e.
pregnant	30	58.6	±2.78	24.99	±3.02
non- pregnant	27	57.9	±3.28	26.28	±2.86
males	38	64.7	±3.78	29.37	±3.98

There were significant differences between the body temperatures of the three categories of animals recorded in the field (ANOVA: $F_{2, 92} = 9.90$; $P = 0.0023$). The data show that, in the field, mean body temperature of pregnant females was approximately 10% lower than the mean body temperature of the non-pregnant females, and approximately 15% lower than that of males. A *post hoc* Fisher's LSD test demonstrated that the significant difference in field body temperatures lay between the pregnant and non-pregnant ($P < 0.05$) females, pregnant females and males ($P < 0.00001$), and non-pregnant females and males ($P < 0.005$). This suggests that female *Niveoscincus microlepidotus* select a lower body temperature in the field than males, and non-pregnant females select lower body temperatures than non-pregnant females as demonstrated in the laboratory.

3.4 DISCUSSION

In reptiles, it has been found that there is a very limited range of optimal incubation temperatures necessary for the proper development of the foetus (Beuchat, 1988). Depending on the species, this may be lower or higher than the optimal temperatures for various physiological functions of the mother (Beuchat, 1988). The ability to behaviourally regulate body temperature in viviparous lizards such as *Niveoscincus microlepidotus* allows females to select body temperatures that are optimal for embryonic development. Temperature can strongly affect the rate of development of reptilian embryos (Muth, 1980; Beuchat and Ellner, 1987), and a change in

thermoregulatory behaviour during pregnancy is regarded as an adaptive shift to maximise neonate survival and fitness (Garrick, 1974; Beuchat and Ellner, 1987).

A pregnant female may need to maintain a body temperature that is a compromise between her own optimal temperatures, and those necessary for embryonic development (Beuchat and Ellner, 1987; Schwarzkopf and Shine, 1992). This compromise is critical, for if the temperature is either too high or too low it can result in reduced viability or abnormalities of the young (Beuchat, 1988). For example, in *Sceloporus jarrovi* gestation at the optimal gestation temperature of 32 °C resulted in 95% viability of offspring, with no obvious morphological or behavioral abnormalities. However, an increase or decrease of as little as 3 °C led to over 50% mortality or congenital birth defects for both the higher and lower temperatures (Beuchat, 1988).

It was found that pregnant *Niveoscincus microlepidotus* females decrease the upper thermal setpoints and preferred body temperatures (Kik, 1998; and current study). Thus, in order to maintain a lower eccentric body temperature, pregnant *Niveoscincus microlepidotus* avoid extreme temperatures and rarely allow their body temperature to reach temperatures above 31 °C. Average maximum temperature values in pregnant females were 3-4 °C lower than those achieved in non-pregnant females. This suggests that high temperatures (at least those ≥ 31 °C) may be damaging to the embryos of *N. microlepidotus*.

Higher body temperatures during pregnancy are generally thought to increase the rate of embryonic development (Vinegar, 1974; Muth, 1980; Beuchat, 1988; Schwarzkopf and Shine, 1991; Van Damme *et al.*, 1991), whereas lower body temperatures slow gestation (Daut and Andrews, 1993). *Niveoscincus microlepidotus* retains the embryos through hibernation until the following spring or early summer. Therefore, as long as embryos can develop to the final growth stage (as described by Defaure and Hubert, 1961) before the end of the activity season, there would be no advantage in rapid development of young. This suggests that it may be prudent to decrease maternal body temperature to slow development until close to hibernation; thus

reducing any cost incurred by carrying the fully developed neonate, such as a decrease in locomotory performance (Chapter 4) or increase in metabolic rate (Chapter 5).

The physiological optimum of non-pregnant female *Niveoscincus microlepidotus* has been suggested to be approximately 26 °C (Melville, 1994). It has been shown that pregnant females select upper body temperatures that are lower than this temperature (Kik, 1998; current study). However, there is no evidence of a decrease in the lower thermal setpoint (Kik, 1998; current study). It can be assumed that optimum temperature for embryonic development is closely correlated with selected body temperatures. Therefore, it can be concluded that the thermal optimum for the embryonic development in *N. microlepidotus* lies within the range 22 – 24 °C. Temperatures other than this presumably adversely affect development of the embryos. Mathies and Andrews (1997) recorded the same phenomenon in pregnant females of *Scleroporos jarrovi*. This may be important for a pregnant female occupying an alpine habitat in which sudden and frequent changes in temperature often occur. It also allows pregnant females to maintain maximal activity levels in colder conditions, permitting easier maintenance of resource levels needed to ensure survival and growth of the neonate and mother.

The maintenance of upper and lower setpoints in *Niveoscincus microlepidotus* may also have important consequences for microhabitat selection. The body temperatures of pregnant females that require the cessation of basking are approximately 2-3 °C below those of non-pregnant lizards (Kik, 1998; current study). It has been shown that there may be some temperature effect on performance (Chapter 4). These changes may have an effect on what constitutes a suitable basking site. Pregnant female *N. microlepidotus* appear to select more cryptic basking sites that are closer to safety, and usually have at least some leaf cover (Kik, 1998; pers. obs.). Such sites are presumably cooler and more protected than the sites selected by non-pregnant females which choose to bask on the exposed upper surfaces of boulders, allowing maximum sun light exposure (Kik, 1998). If pregnant females utilised similar basking sites to non-pregnant females, they would reach their upper thermal setpoint rapidly, and thereby may reduce total time spent at optimal temperatures. However, the increased

weight of a female due to the clutch mass has shown to significantly decrease the heating rate. Therefore, the pregnant female must devote a larger amount of time to thermoregulation, in order to maintain an optimal body temperature (Shine, 1980; Avery, 1982; Schwarzkopf and Shine, 1992). It appears that pregnant *N. microlepidotus* reduce predation risk, and optimise embryonic development by changing their basking sites, rather than the time spent basking (Kik, 1998). However, no direct measurements of basking times in the field were carried out in this study.

CHAPTER 4

PERFORMANCE ABILITIES

4.1 INTRODUCTION

A basic tenet of life-history theory is that trade-offs exist between major components of fitness; reproduction in particular is associated with major costs (Brodie, 1989). Some of these costs may be physiological, and may manifest as a decrease in locomotory performance (Shine, 1980). For many terrestrial animals this may affect maximum sprint speed, climbing performance, stamina and jumping, all of which are essential abilities for navigating through habitat during daily activities. This chapter will concentrate on locomotory performance changes associated with pregnancy in *Niveoscincus microlepidotus*.

The unusually long gestation of *Niveoscincus microlepidotus* requires the pregnant female to carry an increasing load as the clutch grows; this may have an escalating impact on her performance ability. In *N. microlepidotus*, the total body mass of pregnant females increases by approximately 25 - 30% during gestation (pers. obs.). In most animals, carrying a heavy load may result in distinct changes in locomotory ability. In reptiles, performance has been shown to be affected by a number of factors including reproductive status and stage, recent ingestion of food, and body temperature (Shine, 1980; Bauwens and Thoen, 1981; Brodie, 1989; Van Damme *et al.*, 1989; Cooper *et al.*, 1990; Sinervo *et al.*, 1991; Cooper, 1997a,b). Decreased mobility is, therefore, believed to be a particularly important potential cost associated with gestation. This may be due to the effects it will have on foraging and predator avoidance (Shine, 1980; Garland, 1985; Seigel *et al.*, 1987).

Niveoscincus microlepidotus is a locomotory "generalist" and moves through its habitat by a mixture of running, jumping and climbing (Melville, 1998). Any or all of these forms of mobility may be affected by pregnancy. These changes are difficult to assess accurately in the field; however, laboratory investigation of performance capacity is now well established as an acceptable, quantifiable measure of an animal's

performance (e.g. Shine, 1980; Huey *et al.*, 1981, Huey, 1982). Thus, sprint speed, endurance, climbing, and jumping ability provide ecologically relevant measures of performance. Consequently, this chapter extends the work done by Kik (1998) who examined the ecological and behavioural responses associated with pregnancy in *Niveoscincus microlepidotus*; thus in this Chapter the following questions were addressed.

- Is maximum sprint speed reduced during pregnancy?
- Is endurance reduced during pregnancy?
- Is jumping ability reduced during pregnancy?
- Is climbing ability reduced during pregnancy?
- What effect might any such changes have on the behaviour of *Niveoscincus microlepidotus*?

4.2 MATERIALS AND METHODS

4.2.1 COLLECTION AND PREPARATION OF ANIMALS

As discussed in Chapter 2, a major advantage of *Niveoscincus microlepidotus* is the concurrent availability of pregnant and non-pregnant animals. However, the three desired pregnancy stages were not always equally available; for example in late spring animals tended to be either close to parturition or in the very early stages of pregnancy. Animals in different reproductive stages were caught at each collection. However, as far as possible, comparable numbers of animals at each “stage” were collected.

Approximately 10 - 18 pregnant and non-pregnant females were collected at each occasion to be tested. All lizards were collected from the Mt Wellington field sites (see Chapter 2). Animals were housed as described in Chapter 2.3.

For each performance experiment the body temperature of experimental lizards was controlled to 24, 26 or 28 °C (± 1 °C) by placing the animals in a watertight metal container immersed in a Techne™ Temperor™ water bath. Each lizard was acclimated to the test temperature for 30 minutes before each trial. This method of raising the lizard's body temperature has advantages over many other methods, which involve heating from one surface, as control is more accurate and more stable temperatures are obtained. This will subsequently be referred to as standard heating procedure. For all studies each animal was tested at only one experimental temperature.

4.2.2 SPRINT ABILITY

There is a significant relationship between sprint speed and temperature in reptiles (Huey and Stevenson, 1979; Huey, 1982; Huey and Bennett, 1987; Avery and Bond, 1989; Brodie, 1989; Van Damme *et al.*, 1989). Sprint speed is maximised as temperature approaches an optimum, after which point performance begins to decline (Huey and Bennett, 1987; Melville 1994). *Niveoscincus microlepidotus* was tested for sprinting ability at 3 temperatures: “optimal performance temperature” 26 °C (Melville 1998) and 2 °C above and below this “optimum” (24, 28 °C). Females in four reproductive states (non-, early-, middle-, late-pregnancy) were tested.

A heated 1.5 m long “race track” with a metal bottom, lined with fine sandpaper to allow traction, available from previous studies was used in this trial. Temperature probes and 120 watt spotlights were attached to the underside of the track to allow the track temperature to be controlled to ± 1 °C. Three photodiodes, at 50 cm intervals, linked to a Macintosh computer, allowed sprint times to be recorded (Figure 4.1). Each run provided two estimates of speed, and each lizard was run three times at the same temperature with a minimum rest of 30 minutes between trials. The fastest recorded time (cm sec^{-1}) was considered the maximal speed at that temperature. Animals were then released at the approximate spot from which they were collected.

4.2.3 CLIMBING ABILITY

Climbing ability was measured as the time to climb a 50 cm long x 2.5 cm diameter rod. Each animal was warmed using the standard procedure, to one of the three test temperatures (24, 26, 28 °C). The round wooden rod, roughened with coarse sandpaper to increase traction, was placed at a 45° angle in a 150 x 150 x 30 cm box with a 5 cm layer of sand substrate. Speed was measured over the 50 cm distance using two stopwatches and two observers to minimise error. The mean of the two recorded times was considered the true time to transverse the distance. Speed was calculated as centimetres per second. Each animal was tested three times at the desired test temperature with a minimum of 30 minutes rest between trials. The fastest time recorded was considered the maximal climbing speed for that temperature. Animals were excluded from the data set if they refused to climb, fell off, or jumped from the rod prior to reaching the end.

4.2.4 JUMP DISTANCE

A large box 150 x 150 x 30 cm, was used to test jumping ability. The floor was covered with a 5 cm layer of sand substrate and in the centre was located an upright 15 cm tall tower with a square wooden platform (10 x 10 cm). A smooth plastic box covered the platform so that animals could not climb down. Sandpaper was glued to the top so that lizards were able to create traction and jump effectively.

Each animal was warmed by standard procedure to the desired test temperature. Trials were conducted by placing lizards in the middle of the platform, and encouraging them to jump by gently tapping their tail. *Niveoscincus microlepidotus* jumps readily and minimum encouragement was needed. Each animal was tested three times with a minimum of 30 minutes rest between trials. Jumping distance was defined as the longitudinal distance, along the ground, from the impact print to the platform's base equivalent to the spot of launch. Distance was measured to ± 0.5 cm. The furthest

jump of the three trials was considered the maximum jump distance for that temperature.

4.2.5 ENDURANCE CAPACITY

A treadmill, 50 x 20 cm, with a neoprene belt was used to test endurance capacity (Figure 4.4). Lizards were heated to one of the test temperatures using the standard procedure. A small motor regulated the treadmill speed to 6.90 cm sec^{-1} which was determined to be most suitable because at this speed animals moved willingly and with no visible signs of distress (Melville 1998). Testing was carried out in the lizard housing facility where the air temperature was approximately 12°C . A lamp with a 40 watt bulb was placed directly over the running surface to maintain the animal's body temperature. The trial was terminated when an animal stopped and refused to move after several gentle taps to its tail, and the total time was recorded. Due to the potential stress of running to fatigue each animal was tested only once.

4.2.6 DATA ANALYSIS

Analyses were performed using the SAS System for Windows[®] v6.12. Homogeneity of variances was tested by visual inspection of plots of group standard deviations versus group means. Normality of data distribution was addressed by visual inspection of the log-normal plot of estimated residuals and the plot of estimated versus predicted residual values. Where appropriate data were log transformed to adhere to the assumptions of ANOVA (see Section 4.3).

For each performance behaviour the following sequence of analyses was conducted. First, a regression analysis was performed to test the null hypothesis that size (SVL) has an effect on performance. Rejection of this hypothesis allowed the analysis of data without the need to use SVL as a co-variate.

It was also necessary to test the null hypothesis that there was no seasonal effect on locomotory performance. The control data (non-pregnant animals) were regressed

against season to test for any seasonal effect on performance. The rejection of this hypothesis allowed the control data to be pooled in order to compare differences between reproductive states (non-, early-, mid-, and late-pregnant). Because pregnant animals were found at different stages of pregnancy through the collection periods, and were then pooled by stage and not season collected, it was not necessary to first evaluate any seasonal effects (see Section 4.2.1).

A two-way ANOVA was then used to test the null hypothesis that reproductive state and temperature had no effect on performance. Where significant differences were found, a series of one-way ANOVAs were performed against reproductive state or temperature with corresponding Fisher's LSD *post hoc* tests, to identify significant groups within treatments. Where the two-way ANOVAs yielded significant interaction effects, the interactions were evaluated by visual inspection of the graphed results.

4.3 RESULTS

4.3.1. SPRINT SPEED

Melville (1994) showed that sprint performance initially increased with temperature in non-pregnant *Niveoscincus microlepidotus* females, then reached a broad and stable plateau until a point was reached where the performance declined. The three test temperatures used in this study were within this plateau, prior to the decline in performance.

A total of 296 pregnant and non-pregnant females was used in this experiment. A preliminary regression analysis determined that SVL had no significant relationship with sprint speed performance (Table 4.1).

Table 4.1 Mean SVL (\pm s.e.) and the result of regression analysis of SVL against sprint speed in *N. microlepidotus* tested at four reproductive states; non-, early-, mid-, and late-pregnant.

State	n	mean SVL	s.e.	ANOVA df	F	P
early-pregnant	60	58.7	3.04	1, 59	0.586	0.4472
middle-pregnant	42	57.9	2.94	1, 40	3.409	0.0722
late-pregnant	41	60.6	3.14	1, 39	2.098	0.1564
non-pregnant	153	58.5	2.43	1, 151	0.397	0.5296

A regression analysis determined that there was no significant relationship between speed and season in non-pregnant females (ANOVA: $F_{1,151} = 0.356$; $P = 0.5586$). Therefore all performance data obtained from the pregnant animals were compared to the performance of the non-pregnant females tested at the same temperature regardless of season (Figure 4.1).

The data suggest that the performance of pregnant females mirrors the performance of the non-pregnant females in the early stages of pregnancy as well as at the lower test temperature. However, the performance of pregnant females appeared to decline when tested at higher temperatures during middle and late pregnancy.

A two-way ANOVA was used to evaluate these results. Significant differences were observed between reproductive status and maximal sprint speed (ANOVA: $F_{3, 284} = 4.01$; $P = 0.0498$) and temperature and maximal sprint speed (ANOVA: $F_{2, 284} = 27.10$; $P = 0.0001$). There was also a highly significant interaction effect of temperature and stage of pregnancy on maximal sprint speed (ANOVA: $F_{6, 284} = 8.29$; $P = 0.0001$).

A series of one-way ANOVAs performed on temperature and *post hoc* Fisher's LSD tests, indicated that only high temperature (28 °C) had a significant effect on sprint speed ($P < 0.005$). A similar analysis of reproductive state showed that late pregnancy stage accounted for the significant effects of this variable ($P < 0.0005$). This suggests

that the interaction effect was the result of a significant decrease in sprint performance for late stage pregnant females at higher temperature. This was confirmed by visual inspection of Figure 4.1.

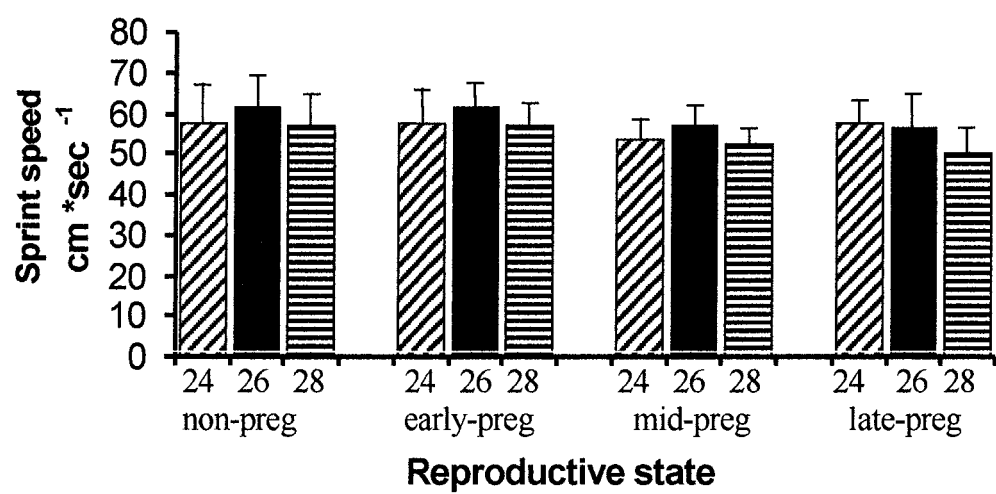


Figure 4.1 The mean maximal sprint speed for non-, early-, mid-, and late-pregnant *Niveoscincus microlepidotus* females at the three test temperatures (24, 26, 28 °C)

The significant decrease in sprint speed for the late pregnant females suggests that, for these animals, the upper temperature at which a decline in sprint speed occurs has shifted down compared to the equivalent temperature for non-pregnant females.

4. 3. 2. CLIMBING PERFORMANCE

In this experiment 271 pregnant and non-pregnant females were used. The mean SVL is summarised in Table 4.2. Regression analysis was used to confirm that body length (SVL) did not have a significant relationship to climbing performance for any reproductive state tested.

Table 4.2 Mean SVL (\pm s.e.) and the result of regression analysis of SVL against climbing performance in *Niveoscincus microlepidotus* tested during non-, early-, mid-, and late-pregnant states

State	n	mean SVL	s.e.	ANOVA df	F	P
early-pregnant	45	59.8	2.49	1, 43	0.956	0.334
middle-pregnant	32	58.6	3.09	1, 30	2.154	0.154
late-pregnant	42	60.4	2.97	1, 40	3.459	0.756
non-pregnant	152	55.9	7.43	1, 150	0.100	0.753

There was no significant seasonal effect on climbing ability in the non-pregnant females (ANOVA: $F_{1,108} = 0.0536$; $P = 0.8196$), so all performance data obtained from pregnant animals were compared to the pooled performance of the non-pregnant females tested at the equivalent temperature for the entire experiment.

A two-way ANOVA was used to evaluate the data. There was a significant difference in maximal speed associated with reproductive state (ANOVA: $F_{3, 260} = 7.53$; $P = 0.0001$) and with temperature (ANOVA: $F_{2, 260} = 5.23$; $P = 0.0059$). A significant interaction effect of temperature and stage of pregnancy on maximal climbing speed was shown (ANOVA: $F_{6, 260} = 2.29$; $P = 0.0491$).

A series of one-way ANOVAs were performed on temperature against climbing speed with Fisher's LSD *post hoc* tests where appropriate. These demonstrated that only the high test temperature had a significant negative effect on climbing speed ($P < 0.0001$). The same analysis on reproductive state showed that the late pregnancy group accounted for the significant effects of this variable ($P < 0.003$). This suggests that the interaction effect was a result of a significant decrease in climbing performance in late stage pregnant females at higher temperature. This was confirmed by visual inspection of Figure 4.2.

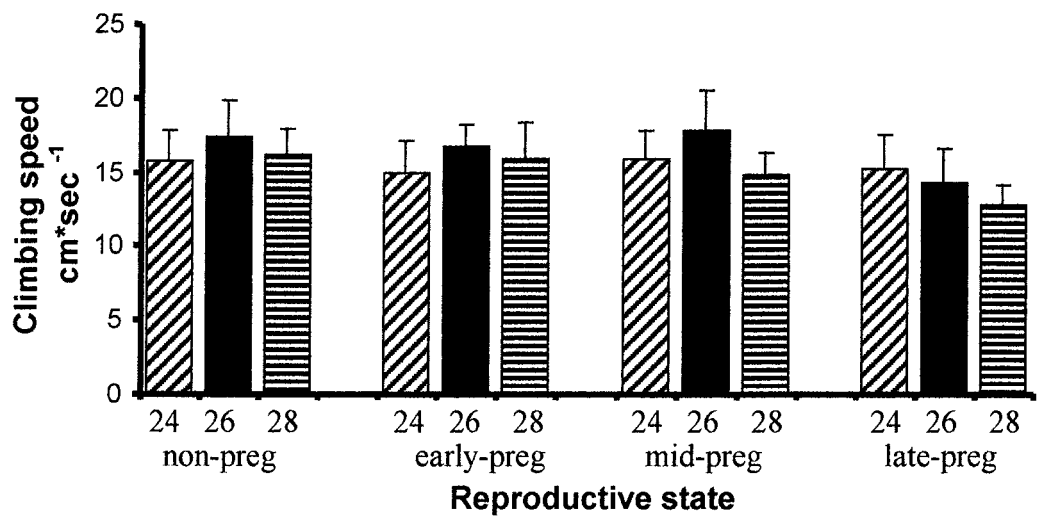


Figure 4.2 Mean climbing performance for non-, early-, mid-, and late- pregnant *Niveoscincus microlepidotus* females at the three test temperatures (24, 26, 28 °C)

The data show that the performance of the pregnant females reflects the performance of the non-pregnant females when tested at 24 and 26 °C. However, during the middle stages of pregnancy, the performance of the pregnant females declines when tested at the higher temperature 28 °C, and declines dramatically during the late stages of pregnancy (Figure 4.2)

It can therefore be surmised that pregnancy does have an effect on the climbing ability of *Niveoscincus microlepidotus*. This is further evidence that there is a significant temperature effect on maximal performance during pregnancy. The data suggest that, as with sprint capacity, the optimal performance temperature for climbing ability may have been shifted down during pregnancy.

4.3.3 JUMPING PERFORMANCE

It is an intuitive assumption that the increase in weight during pregnancy will have a profound effect on the ability to jump, and as with sprint speed and climbing ability,

temperature can be expected to correlate with jumping performance. For this experiment 258 pregnant and non-pregnant females were used.

Table 4.3 Mean SVL (\pm s.e.) and the result of regression analysis of SVL against jumping ability in *Niveoscincus microlepidotus* tested during non-, early-, mid-, and late-pregnant states

State	n	mean SVL	s.e.	ANOVA		
				df	<i>F</i>	<i>P</i>
early-pregnant	42	59.8	2.49	1, 40	0.013	0.9100
middle-pregnant	39	58.6	3.09	1, 37	0.045	0.8327
late-pregnant	42	60.4	2.97	1, 40	1.902	0.1755
non-pregnant	135	57.0	2.81	1,133	0.978	0.3246

A preliminary analysis was carried out to determine whether body length (SVL) was a significant variable. No significant relationship was found between performance ability and body length (SVL) within either the three pregnant groups or the non-pregnant group (Table 4.3).

Regression analysis also determined that there was no significant seasonal effect on jumping performance of non-pregnant females (ANOVA: $F_{1, 133} = 0.978$; $P = 0.3425$). Therefore, all performance data obtained from pregnant animals were compared to the pooled performance of the non-pregnant females tested at the equivalent temperature for the entire experiment.

A two-way ANOVA revealed that there were significant differences associated with reproductive states in jumping performance (ANOVA: $F_{3, 249} = 4.63$; $P = 0.0036$) and with temperature in jumping performance (ANOVA: $F_{2, 249} = 7.17$; $P = 0.0009$). There was also a significant interaction effect between temperature and reproductive states on maximal jumping ability (ANOVA: $F_{6, 249} = 4.92$; $P = 0.0059$).

A series of one-way ANOVAs performed on temperature, with subsequent Fisher's LSD *post hoc* tests against jumping performance, demonstrated that only high temperature (28 °C) had a significant effect ($P < 0.005$). A similar analysis on reproductive state showed that differed significantly from all other reproductive states ($P < 0.00001$). This suggests that the interaction effect was a result of a significant decrease in jumping performance in late stage pregnant females at higher temperature. This was confirmed by visual inspection of Figure 4.3.

The data show that there was no difference in the jumping performance of the pregnant females at 24 °C throughout the experiment. However, in the late pregnancy group at 26 °C there was a slight but non-significant decrease in performance, and the 28 °C group showed a significant decrease in performance Figure 4.3.



Figure 4.3 Mean maximal jumping performance for non-, early-, mid-, and late-pregnant *Niveoscincus microlepidotus* females at the three test temperatures (24, 26, 28 °C)

4.3.4 ENDURANCE PERFORMANCE

A total of 237 pregnant and non-pregnant females was used for this experiment (Table 4.4). A preliminary regression was performed in order to test if body length had an

effect on endurance. There was no significant relationship found between body length and endurance for any experimental group.

Table 4.4 Mean SVL (\pm s.e.) and the result of regression analysis of SVL against endurance capacity in *Niveoscincus microlepidotus* tested during non-, early-, mid-, and late-pregnant states

State	n	mean SVL	s.e.	ANOVA		
				df	F	P
early-pregnant	40	60.2	3.02	1, 38	0.248	0.6209
middle-pregnant	42	57.9	2.49	1, 40	1.879	0.1781
late-pregnant	41	61.1	3.17	1, 39	0.362	0.5510
non-pregnant	114	58.13	3.13	1, 112	1.112	0.2740

A regression analysis determined that there was no significant seasonal effect associated with endurance capacity in the non-pregnant females (ANOVA: $F_{1,113} = 0.361$; $P = 0.5615$). Therefore, all performance data obtained from pregnant animals were compared to the pooled performance of the non-pregnant females tested at the equivalent temperature for the entire experiment.

A two-way ANOVA was used to evaluate the data. There was a significant effect of reproductive status (ANOVA: $F_{3, 112} = 95.56$; $P = 0.0001$) and temperature (ANOVA: $F_{2,112} = 10.08$; $P = 0.0001$) on endurance capacity. There was also a significant interaction effect of temperature and reproductive stage on maximal endurance capacity (ANOVA: $F_{6, 112} = 2.19$; $P = 0.0481$).

A series of one-way ANOVAs for each test temperature against endurance capacity with a Fisher's LSD *post hoc* tests, demonstrated that both of the higher temperatures (26 and 28 °C) had a significant effect on endurance capacity ($P < 0.005$; $P < 0.0001$ respectively). Analysis of reproductive status showed that pregnancy alone accounted for the significant decrease in endurance capacity ($P < 0.005$). This suggests that the interaction effect was a result of a significant decrease in endurance capacity in all pregnant groups at higher temperatures. This was confirmed by visual inspection of Figure 4.4.

The data show a decrease in endurance capacity between the non-pregnant females and all the pregnancy stages, regardless of test temperature. There also seems to be a further decrease in endurance capacity within each stage of pregnancy with increasing temperature (Figure 4.4). The data strongly supports the argument that temperature has a significant effect on all locomotory performances during the later stages of pregnancy in this species.

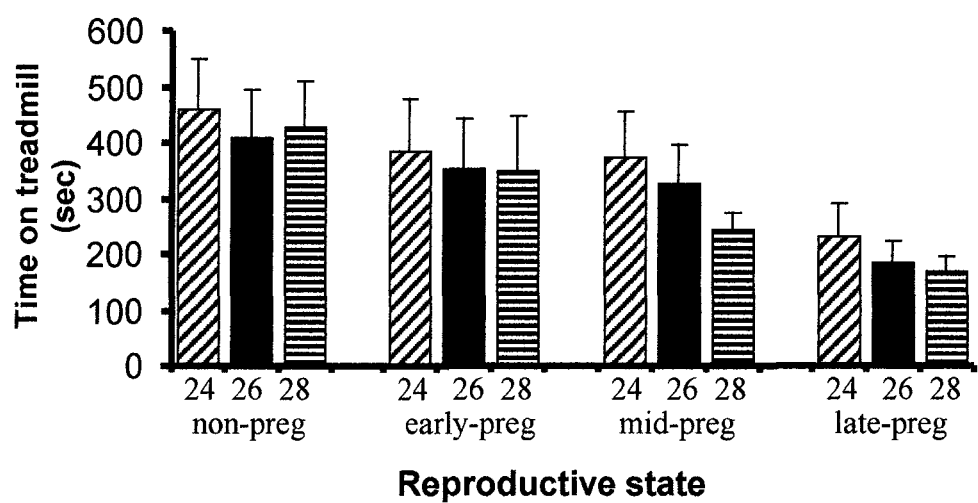


Figure 4.4 The mean maximal endurance capacity for non-, early-, mid-, and late- pregnant *Niveoscincus microlepidotus* females at the three test temperatures (24, 26, 28 °C)

4.4 DISCUSSION

The results show that there are changes in locomotory performance with temperature in adult female *Niveoscincus microlepidotus* during pregnancy that do not appear in non-pregnant females. Pregnant females showed no significant differences in sprint, climbing and jumping performance when compared to non-pregnant females, at temperatures below those previously described as optimal for this species (Melville, 1994; Kik, 1998; Chap. 3). However there was a significant reduction in performance during late pregnancy at temperatures that were slightly higher than this optimal

performance temperature. Pregnant females also showed a significant decrease in endurance capacity during pregnancy (Kik, 1998), which was most apparent during the later stages and was exaggerated at temperatures above the performance optima. These results indicate that, for *N. microlepidotus*, changes in performance may be related to pregnancy and temperature, suggesting that the pregnant female may have a temporary physiological shift in optimal performance temperature.

Studies of small lizards have suggested that carrying loads such as a clutch or food is associated with decreased mobility (Shine, 1980; Bauwens and Thoen, 1981; Brodie, 1989; Cooper *et al.*, 1990; Sinervo *et al.*, 1991; Cooper, 1997a) and endurance capacity (Cooper *et al.*, 1990; Sinervo *et al.*, 1991). A decrease in mobility during gestation has also been shown in two closely related species: *Niveoscincus ocellatus*, and *Niveoscincus metallicus* (Wapstra, 1998; Kik, 1998; C. McCoull pers. comm.). Wapstra (1998) reported that, although *N. ocellatus* and *N. microlepidotus* are similar in size, *N. ocellatus* is able reach speeds ($\sim 120 \text{ cm sec}^{-1}$) twice those of *N. microlepidotus* ($\sim 60 \text{ cm sec}^{-1}$; Kik, 1998); this value may be reduced by as much as 25% in animals close to parturition. The performance of *N. metallicus* decreases at mid pregnancy, and continues to decline until late pregnancy (C. McCoull pers. comm.). The performance abilities of pregnant *N. microlepidotus*, therefore, appear to be somewhat atypical for the genus. The reasons for this response are not immediately apparent, though it may be that this species is a locomotory generalist compared to other species of *Niveoscincus*, and does not show behavioural or morphological specialisation for any particular locomotory mode (Melville, 1998).

As discussed in Chapter 3, *Niveoscincus microlepidotus* shows significant thermoregulatory changes during pregnancy. The performance of pregnant *N. microlepidotus* has been shown to be affected by high temperature. Sprint speed, jumping and climbing performance all decline in the later stages of pregnancy, once the temperature reached a point higher than the optima determined for non-pregnant *N. microlepidotus* (26 °C; Melville, 1994). However, the data show that the pregnant female is able to perform significantly better at a temperature at or below this than at a

slightly higher temperature (~28 °C). This suggests that the pregnant *N. microlepidotus* may temporarily lower its optimal temperature.

It has been shown that pregnant *Niveoscincus microlepidotus* select lower body temperatures presumably to optimise embryonic development. In other species a reduction in maternal body temperature has been shown to have a significant effect on performance ability (Shine, 1980). However, this investigation shows that, at temperatures lower than the thermal optima previously described for non-pregnant animals (24 °C), there is no significant decrease in performance (Melville, 1994). It has also been shown in Chapter 2 that pregnant *N. microlepidotus* rarely allow their body temperatures to rise above 26 °C. This suggests that *N. microlepidotus* is able to temporarily shift its physiological optimal temperature to ~24 °C. This reduction in physiological thermal optima does not appear to have been demonstrated in other lizards. It suggests that *N. microlepidotus* is able to adapt physiologically to the lower temperature needed for proper embryonic development, thereby minimising much of the thermal cost of reproduction.

Escape behaviour in *Niveoscincus microlepidotus* includes climbing, jumping off rocks, and running along “predetermined” escape routes (Schwarzkopf and Shine, 1992; Brana, 1993)). The ability to run at maximum speed is likely to be relevant when escaping predation. Although *N. microlepidotus* has shown no decrease in performance while pregnant (Kik, 1998; current work), the burden of carrying a clutch is thought to be the most important factor affecting the female’s survival during reproduction (Shine, 1980). Animals moving through an open habitat with little vegetation and shelter may be associated with a higher risk of predation, as movement is frequently an important cue used by visual predators such as birds and snakes to detect prey (Curio, 1976). Pregnant female *Niveoscincus microlepidotus* clearly shows a decrease in endurance capacity compared to the endurance of non-pregnant females and this may have implications for behaviour (Kik, 1998; current study). Increased load is energetically costly, especially if an animal is required to run quickly over relatively long distances. *Niveoscincus microlepidotus* is an active forager that feeds primarily on insects (R. Swain pers. comm.). In the laboratory the

feeding behaviour of pregnant female *N. microlepidotus* has been observed to be as “aggressive” as that of non-pregnant females behaviour (pers. obs.). The pregnant female’s feeding behaviour in the wild is unknown. However, it has been shown that pregnant females spend significantly more time basking and less time actively foraging (Melville and Swain 1997a; 1999). This suggests that the foraging strategy of pregnant *N. microlepidotus* changes during pregnancy in the field. Melville and Swain (1997a) reported that home range of pregnant females is significantly smaller than non-pregnant females and there is no evidence of decrease food intake during pregnancy. Thus, the decrease in endurance capacity may not have significant implications on foraging ability. However, a decrease in endurance capacity during pregnancy may have important implications for anti-predatory responses.

Pregnant *Niveoscincus microlepidotus* are unable to sustain activity for as long as their non-pregnant counterparts. Consequently, pregnant females may have to be cautious of the distance between basking and foraging sites to suitable safe areas such as vegetation cover or cracks between rocks (Kik 1998). As discussed in Chapter 3, there is evidence that *N. microlepidotus* becomes more cryptic during pregnancy as occurs in some other viviparous species of lizards (Avery, 1975; Bauwens and Thoen, 1981; Lima and Dill, 1990; Van Damme *et al.*, 1990; Kik, 1998). It has also been shown that pregnant females in this species select basking sites that are less exposed and closer to safety (Kik 1998). Bauwens and Thoen (1981), Schwarzkopf and Shine, (1992), and Martin and Lopez (1995) also found significant decrease in endurance capacity, which were suggested to increase the risk of predation. They suggested that impaired endurance capacity may not translate into reduced survival as the pursuit phase of predation rarely determines the outcome. Most of the efficiency of predation is gained or lost in the detection phase. Thus pregnancy may lead to increased predation through an increase in detection risk rather than through a reduction in mobility (Schwarzkopf and Shine, 1992). Consequently, gravid females may use behavioural modifications to decrease the risk of predation.

It can be concluded that pregnant *Niveoscincus microlepidotus* females are able to temporally shift physiological optimal performance temperature to match the lowered

body temperature needed for the optimal embryonic development. Thereby decreasing the risk of predation, possibly increasing foraging success, and maximising normal behavioural patterns and consequently minimising any performance costs associated with pregnancy.

CHAPTER 5

METABOLISM

5.1 INTRODUCTION

Metabolism is the total of all the biochemical processes within an organism. It is important to understand the metabolic requirements of any species as these affect many aspects of its physiological ecology including microhabitat selection, patterns of life-histories, and population dynamics (Beuchat and Vleck., 1990; Beaupre, 1992; Beaupre, *et al.*, 1993).

The basal metabolic rate of an endothermic animal is the minimal metabolic rate at rest. The body temperature of an ectotherm varies depending on ambient temperature, and minimal metabolic rate likewise varies with body temperature. Therefore an equivalent value to “basal” is needed to describe the minimal rate of an ectotherm. Standard metabolic rate (SMR) has been defined as the lowest metabolic rate of an ectotherm at rest, post-thermoregulatory, and undisturbed at a notable temperature (Wood *et al.*, 1978). Maintenance of a sufficient SMR is critical to the survival of the individual (Wood *et al.*, 1978; Niewiarowski *et al.*, 1992). The SMR of a reptile is sensitive to many factors, including mass of the animal, body temperature, diel cycles, sex, feeding state, and reproductive state (Beaupre, 1992; Beaupre *et al.*, 1993). Variations in maintenance requirements will greatly affect the resources available for growth, reproduction, storage, and activity, and therefore may result in changed survival or fecundity (Beaupre *et al.*, 1993).

Viviparous animals may experience an increase in metabolic demand as the foetus grows. This increase may be due to one or all of the following: an increase in facilitated gas exchange, transfer of organic and inorganic nutrients allotted to the foetus, and the removal of foetal nitrogenous waste (Beuchat and Vleck., 1990; Beaupre, 1992; Beaupre *et al.*, 1993). Unlike eutherians, the majority of viviparous

lizards are lecithotrophic and have to facilitate the embryos with little more than water and gas exchange during gestation. Therefore, they may not incur the substantial increased costs associated with organic nutrient transfer to the foetus (DeMarco and Guilette, 1992). Published research on the metabolic cost of reproduction in viviparous lizards and snakes has reported that the standard metabolic rate (SMR) is greater than the sum of the maternal somatic and the clutch's metabolism (Wood *et al* 1978; Beuchat and Vleck, 1990; Beaupre, 1992; Beaupre *et al.*, 1993; DeMarco, and Guilette 1992; DeMarco, 1993). It is believed that this increase in SMR may be caused by maternal adaptations to pregnancy, and it has been termed the *maintenance cost of pregnancy* (MCP) (Beuchat and Vleck, 1990; Beaupre, 1992; Beaupre *et al.*, 1993). It is believed that MCP results from an increase in facilitated gas exchange and nitrogen waste handling, and fetal metabolic rate. This may have an effect on the resting heart rate and respiration of the pregnant female, thereby increasing standard metabolic rate (Birchard *et al.*, 1984; Niewiarowski *et al.*, 1992). However, Swain and Jones (1997) have shown that a closely related species to *Niveoscincus microlepidotus*, *Niveoscincus metallicus*, is able to transfer organic substrates across its placental membranes to supply the neonate during the later stages of pregnancy. This suggests that there may be additional factors contributing to the observed increase in oxygen consumption, in some species.

5.1.2 OXYGEN CONSUMPTION

Energy consumption can be calculated by measuring the amount of oxygen (O_2) consumed. This is because oxygen cannot be stored in the tissue and is used during all aerobic catabolic processes. The amount of oxygen consumed per unit time has a direct correlation with the energy liberated. The determination of metabolic rate from O_2 consumption is dependent on two assumptions: 1) the energy being used is derived via an aerobic and not an anaerobic pathway; and 2) the amount of energy released is constant, independent of the substrate used (Tortora and Grabowski, 1993). At rest, the first of these assumptions can be confidentially assumed, because most reactions involving substrate utilization are aerobic. The second assumption, however, is not

ideal; the calorific yield per litre of O₂ varies with the type of substrate being oxidized. For example, the oxidation of carbohydrates releases more energy per litre of O₂ than for fats or protein. However, the error involved in this assumption is less than 1%, and because it is usually difficult to identify what substrate is being used, a mean value of 4.82 kilocalories per litre of O₂ consumed is accepted for most calculations based on indirect calorimetry (Tortora and Grabowski, 1993).

5.1.3 ACTIVE METABOLIC RATES

Although SMR is a useful tool for determining a baseline metabolic rate and allows for a general comparison of different ectotherms, these do not provide information about the metabolic costs of essential daily activities. The measurement that best describes the metabolic rates of animals in their natural states is the active metabolic rate (AMR). This represents the average metabolic activity of an animal as it performs its normal activities of daily living. However, variability in temperature, water availability and transport, food quantity and quality, and other experiences of daily living have made it difficult to infer field metabolic rates from laboratory measurements. Until recently, the study of field metabolic rates has been limited by the availability of a small number of suitable techniques. The doubly-labelled water technique is the most frequently applied technique, and has been employed numerous times in ectotherms (Anderson and Karasov, 1981; Merker and Nagy, 1994; Green *et al.*, 1991; Christian *et al.*, 1997). However, use of this procedure requires repetitive blood sampling which is inappropriate in a small species such as *Niveoscincus microlepidotus*. The use of the sodium²² isotope also has been employed however, this technique also relies on repetitive blood sampling. Although Gallagher *et al.*, (1983) described a method of whole-body counting Na²² emissions, this procedure was rejected, due to the long half-life of Na²² (2.62 years), which could be environmentally damaging, and the high cost which could make field measurements economically unfeasible. Peters *et al.* (1995) describe a technique using the rubidium isotope (⁸⁶Rb). Rubidium was shown to mimic the role of intracellular potassium, and therefore its biological elimination rate has a mechanistic correlation with overall

metabolic rate. Peters *et al.* (1995) found that the biological elimination (K_b) of ^{86}Rb is highly correlated ($r^2 = 0.930$) with the K_b derived from the doubly-labelled water technique, which returns a consistent correlation with metabolic rate. The use of Rb^{86} has a number of advantages: it does not require repetitive blood sampling, the isotope has a half life of only 18.7 days, the animal can be whole body gamma-counted, and the isotope is relatively inexpensive. Therefore, it was decided that this technique was appropriate for a trial study using *N. microlepidotus* in indoor enclosures. Rubidium's short half-life makes it an excellent choice for future field use, because this reduces the risk of environmental damage.

In this chapter, the following questions will be addressed.

- What is the standard metabolic rate of *N. microlepidotus* in the laboratory?
- What changes occur in the standard metabolic rate of *N. microlepidotus* during pregnancy?
- Could the rubidium 86 technique be used in the field to evaluate metabolic rates in small free-ranging ectotherms?
- Are there differences in the active metabolic rates between pregnant and non-pregnant female *N. microlepidotus*?
- Are there any observable detrimental effects of the radioisotopes on the neonates?

5.2 MATERIALS AND METHODS

5.2.1 COLLECTION AND PREPARATION OF ANIMALS

All lizards were collected from the Mt Wellington field site (see Chapter 2). Animals were only used once for each indirect calorimetry test. Animals were housed in the

lizard room for use in oxygen consumption trials and for the rubidium experiment, as described in Chapter 2.3.

5.2.2 STANDARD METABOLIC RATE

For each metabolic experiment, approximately 20 pregnant and 20 non-pregnant females were tested at 4 different reproductive states: non-, early-, middle-, and late-pregnancy. The lizards were starved for 24 hours prior to testing and were removed from any heat source at least twenty minutes prior to testing. A digital thermometer was inserted and taped into the subject's cloaca. Animals were placed individually into a sealed container that was equipped with an air intake tube, air egress tube, and thermometer. They were allowed to settle in the container for 10 minutes prior to the commencement of the measurements. The air egress tube was attached to an Ametek Instruments R-2 flow control air pump that pulled air through the system at 50 ml min⁻¹. The air intake was open to the room via a water scavenger, and was warmed by being circulated through a water bath. The egress air was scrubbed for water and CO₂, and the difference in O₂ concentration between the ambient air and the expired air was measured by an Ametek Instruments S-3A/II oxygen analyser and N-37M oxygen sensor. The test chamber was submerged in the water bath which was held at 25 °C (±0.5 °C) by a Ratek Instruments TH1 Thermoregulator™. Once a test had commenced, the animals were not disturbed. Each animal was left in the apparatus until the difference in O₂ concentration per minute had stabilized for a minimum of at least 3 minutes. This stable reading was recorded as the resting O₂ consumption per minute. Any problem with the apparatus or animal was noted and all measurements from that animal were discarded.

The oxygen consumption per minute was calculated by using the formula:

$$VO_2 \text{ (ml/min)} = \Delta O_2 \times \%O_{2(\text{in air})} \times \text{flow rate (ml/min)}$$

where ΔO_2 was defined as the difference between the oxygen concentration in the air entering the respirometer and the oxygen concentration in the air leaving the respirometer. Flow rate was defined as the $\text{ml } O_2 \text{ min}^{-1}$ flowing through the tank.

In order to compare these values between animals, the weight specific oxygen consumption was calculated. This value of $VO_2 \text{ (ml/ g / min)}$ was defined as the SMR at 25 °C.

$$VO_2 \text{ (ml/min/gm)} = \frac{\Delta O_2 \times \text{Flow rate (ml/min)}}{\text{Total body weight (g)}}$$

This value was used to compare the SMR of the pregnant and non-pregnant females across the four reproductive states.

5.2.3 RUBIDIUM TRIALS

For this study 11 pregnant and 11 non-pregnant females were collected from Mount Wellington. The animals were kept in standard conditions for 24 hours, during which time they were; measured (SVL), weighed, and reproductive status was determined by palpation. All pregnant females were estimated to be in mid-pregnancy. Each pregnant female was size matched by SVL to a non-pregnant female and toe-clipped for identification. From the original sample 10 pregnant and 10 non-pregnant animals were injected with 50 μl of 40 $\mu\text{Ci ml}^{-1}$ rubidium in lizard ringer's solution with specific activity of 370 GBq mg^{-1} rubidium⁸⁶ (Peters *et al.*, 1995). The isotope was obtained from Amersham Pharmacia Biotech UK Limited (batch #B-184GB). Intraperitoneal injections were given at a 30° angle into the lateral ventral side of the animals in order to avoid the medial abdominal vein. Once injected, the isotope was allowed to equilibrate in the animal's body for 4 hours, at which point the baseline measurement of radioactivity (t_0) was taken. In order to take measurements and minimise movement during the procedure the lizards were placed in restraints that did not allow movement in any direction. The restraint consisted of a thin plastic sheet

taped snugly around the entire body of the lizard. The restrained animal was placed head-down in the counting well in a Roche MR 252 Automatic Gamma Counter™ for 2 minutes at an energy level setting of 1000-1500 KeV. This procedure did not visibly stress the animals, and returned reproducible results. Readings of background radiation were taken prior to each trial. Two animals (1 pregnant and 1 non-pregnant) were injected with 50µl of lizard ringer's solution in the same manner as described above for use as control subjects and readings were taken in the same manner as for the injected animals. All animals were housed in standard conditions and were tested for radioactivity levels every three days for three weeks, after which readings were taken weekly until background levels (\leq an empty tube) were reached. The animals were kept until they were clearly in late-pregnancy. At this point parturition was induced by an intraperitoneal injection of arginine vasotocin (AVT) in five females. Each animal was given a dose of 2.0 µg AVT in 0.05 ml saline (Guillette, 1979; Fergusson and Bradshaw, 1991). The neonates were examined for any abnormalities, which may have occurred from the use of the radioisotope. All animals were then released near the site at which the mothers were first collected.

In order to calculate the biological elimination rate (k_b); the background emission counts were subtracted from the gross counts of radioactivity to provide a net count for each animal. The net count was corrected for physical decay of the isotope using first order kinetics:

$$C_{(t)} = C_{(t=0)} e^{-(k_p)t}$$

where $C_{(t)}$ is the remaining emission of the isotope at time t , $C_{(t=0)}$ is the initial emission of the isotope at time 0 (initial reading), and k_p is the physical half-life decay constant of the radionuclide:

$$k_p = \ln(2)/T_p$$

where T_p is the half-life of the radionuclide.

The amount of isotope decayed at time T was added to the net count to give the net corrected count. The net corrected count was regressed against time and the slope of this regression line was used to estimate the biological elimination rate (k_b) for each lizard. The biological elimination rate (k_b) of the isotope was then corrected for mass by dividing by the total body weight of the animal, providing an estimate of the biological elimination rate per gram of body weight. This value of $k_b \text{ gm}^{-1}$ was used to compare elimination rates between animals.

5.2.4 DATA ANALYSIS

Analyses were performed on the SAS System for Windows[®] v6.12. Homogeneity of variances was tested by visual inspection of plots of group standard deviations versus group means. Normality of data distribution was addressed by visual inspection of the log-normal plot of residuals and the plot of estimated versus predicted residual values. Where appropriate data were transformed to comply with the assumptions of ANOVA (see Section 5.3).

For each metabolic experiment, the following sequence of analyses was conducted. An initial regression was performed in order to test the null hypotheses that size (SVL) is a significant contributor to variation in standard metabolic rate and the biological elimination rate of the rubidium isotope. The rejection of these hypotheses allowed the comparison of animals of different body size.

It was then necessary to test the null hypotheses that there is no significant difference in standard metabolic rate and in biological elimination rate of the rubidium isotope between any of the reproductive states tested. One-way ANOVAs were used for these analyses to test for significant differences in standard metabolic rate against reproductive states and biological elimination rate of the rubidium isotope against reproductive states. Fisher's LSD *post hoc* tests were used to identify significant groups within treatments when ANOVA results were significant.

5.3 RESULTS

5.3.1 STANDARD METABOLIC RATE

Body weight is a known determinant of the oxygen consumption of an animal so SMR estimates are presented as weight specific oxygen consumption values. The data were log transformed to meet the assumptions of normality required for ANOVA. A total of 73 pregnant and non-pregnant lizards was used for this experiment. A regression analysis showed that there was no significant relationship between SVL and oxygen consumption for any of the reproductive states tested (Table 5.1).

Table 5.1 Mean SVL and regression values of SVL against oxygen consumption for four reproductive states in *Niveoscincus microlepidotus*

State	n	mean	ANOVA			
		SVL	s.e.	df	<i>F</i>	<i>P</i>
early-pregnant	10	55.5	3.71	1, 8	3.561	0.112
middle-pregnant	13	55.9	4.40	1, 11	1.154	0.854
late-pregnant	13	56.9	3.72	1, 11	2.459	0.756
non-pregnant	37	57.4	5.31	1, 35	0.042	0.838

A one-way ANOVA revealed a significant difference in observed SMR between the experimental groups (ANOVA; $F_{3, 69} = 31.98$; $P = 0.0001$). The SMR of the early pregnant females ($0.089 \pm 0.005 \text{ ml g}^{-1} \text{ h}^{-1}$) did not differ from that of the non-pregnant females ($0.088 \pm 0.007 \text{ ml g}^{-1} \text{ h}^{-1}$) tested; however, a Fisher's LSD *post hoc* test indicated that there was a significant difference in SMR between these groups and late-pregnant females ($P < 0.05$). During the middle stages of pregnancy there was an approximately 18% increase in SMR ($0.094 \pm 0.009 \text{ ml g}^{-1} \text{ h}^{-1}$) compared to the non-pregnant and early-pregnant animals, but this was not statistically significant ($P = 0.067$). In the late stages of pregnant female SMR was approximately 24% higher than in non-pregnant females ($0.109 \pm 0.004 \text{ ml g}^{-1} \text{ h}^{-1}$; Figure 5.1). This was highly significant (Fisher's LSD *post hoc* test, $P < 0.0001$). Consequently, it

can be inferred that there is a cost of the maintenance of resting metabolism associated with pregnancy in *Niveoscincus microlepidotus* that increases as embryonic development proceeds. However, it can not be determined if this cost is the result of the maternal metabolism or a combination of both the maternal and foetal metabolisms.

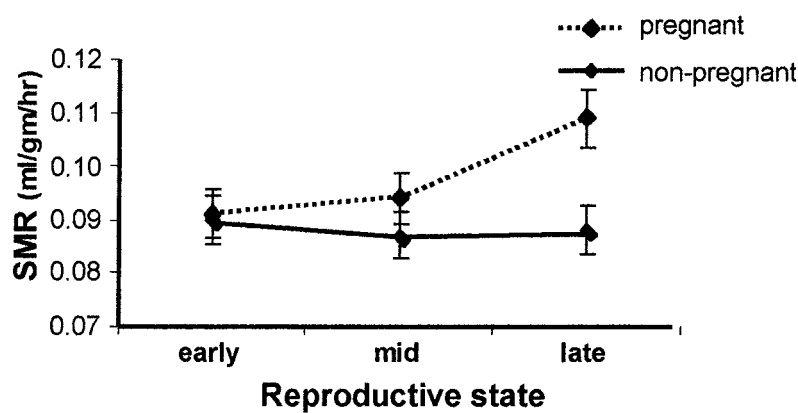


Figure 5.1 The SMR of *Niveoscincus microlepidotus* during three stages of reproduction and the SMR of non-pregnant females during the subsequent season

5.3.2 ACTIVE METABOLIC RATE

Twenty females were used as experimental animals in this study. A preliminary analysis of body length (SVL) against biological elimination rate determined that there was no significant relationship between SVL and k_b for either pregnant or non-pregnant females (Table 5.2).

Table 5.2 Mean SVL and regression values of SVL against active metabolic rate during four reproductive states of *Niveoscincus microlepidotus*

Stage	n	mean SVL	s.e.	ANOVA df	F	P
pregnant	10	58.4	3.37	1, 8	0.305	0.596
non-pregnant	10	57.7	3.03	1, 8	0.003	0.958

One non-pregnant female (#2) demonstrated a k_b that was 3-4 times ($k_b = 4028$) that of the group mean. This animal was clearly a statistical outlier and was removed from the data set. Figure 5.3 shows an example of the biological elimination over 20 days used to estimate k_b .

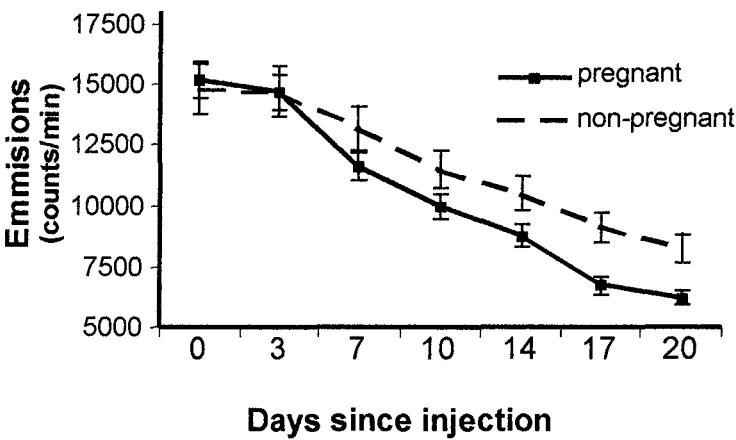


Figure 5.2 Example of biological elimination of the rubidium isotope *Niveoscincus microlepidotus* across 20 days; for pregnant female #4 and non-pregnant female #4

A one-way ANOVA showed that there was a significant difference in k_b between the pregnant and non-pregnant females (ANOVA: $F_{1,17} = 11.78$; $P = 0.0003$). Figure 5.3 shows that in pregnant females the biological elimination rate ($1509.6 \text{ s.e.} \pm 271.4$) is substantially faster than in non-pregnant females ($1172.73 \text{ s.e.} \pm 214.1$). The difference in the biological elimination rate was approximately 29% faster for pregnant females

compared to non-pregnant. The data suggest that there is an increase in active metabolism during pregnancy, is similar to that found for the pregnant females used in the oxygen consumption trial.

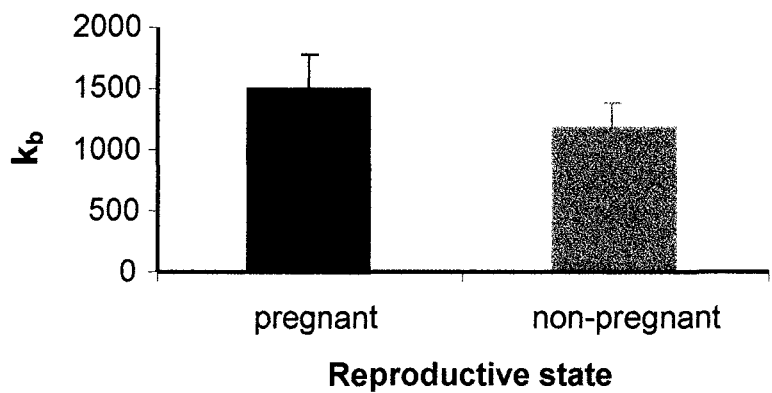


Figure 5.3 The mean biological elimination rate (k_b) of pregnant and non-pregnant *Niveoscincus microlepidotus*

This increase in metabolic rate was confirmed by measuring the oxygen consumption of four pregnant rubidium test animals pre- and post-parturition. These animals also displayed an increased oxygen consumption (mean SMR = 0.113 s.e. \pm 0.008 ml g⁻¹ hr⁻¹) during the late stages of pregnancy, which dropped by 22% within the first two days after parturition (mean SMR = 0.93 s.e. \pm 0.005 ml g⁻¹ hr⁻¹).

An experiment investigating the biological elimination rate of the rubidium isotope was commenced earlier in the development cycle than the data presented here (at approximately stage 29-30). The animals were housed in the outdoor enclosures as described in Chapter 2. Ten animals were killed eight days after initial injection and postmortem dissections were performed. Radioactivity counts were taken from the carcasses, individual organs, and embryos. The results showed that a mean of 62% of the isotope was found in the skeleton and musculature of the mother, and a mean of only 3% of the total emissions was found in the embryos.

The neonates were examined to determine if the radioactivity had any observable negative effects. All five mothers injected had viable clutches of 1 – 3 neonates, and no newborn animals had any external abnormalities. The neonates were held for two weeks and all had started to gain weight. One neonate was captured three weeks later in the upper field site, and exhibited no abnormalities.

5.4 DISCUSSION

This study describes changes in standard and active metabolic rates in adult *Niveoscincus microlepidotus*; data are provided for both pregnant and non-pregnant females. Pregnant females showed significant increases in both SMR, and active metabolic rate. However, increase in SMR was only seen in the later stages of pregnancy, suggesting that the metabolic burden increases as development continues. It was not possible, for logistic reasons, to correlate active metabolic rate with different pregnancy stages.

It has been assumed that the SMR of female lizards during gestation conforms to the standard linear relationship between metabolism and body mass (DeMarco, 1993). However, the data presented here did not support this assumption. The mean SMR of non-pregnant *Niveoscincus microlepidotus* at 25 °C was estimated as $0.088 \text{ s.e.} \pm 0.007 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$. The SMR for non-pregnant females presented here correlates well with the SMR described for other non-pregnant skinks (see Table 5.3). However, the SMR of the pregnant *N. microlepidotus* increases significantly during pregnancy until it reaches a maximum value of $0.109 \text{ s.e.} \pm 0.004 \text{ ml O}_2 \text{ h}^{-1} \text{ g}^{-1}$ just prior to parturition.

The SMR of viviparous lizards appears to be higher than that of non-viviparous species (Table 5.3). This suggests that there may be a correlation between some aspect of a viviparous specie's life-history and a higher metabolic rate regardless of reproductive state. However, the observed increase in oxygen consumption during pregnancy may result from a combination of any or all of the maternal body mass, foetal mass, and maternal physiological changes (Birchard *et al.*, 1984; Beuchat and

Vleck, 1990). Several studies have suggested that increased metabolic rate during pregnancy may be due to an increase in facilitated gas exchange and nitrogen waste handling resulting from embryonic metabolism (Birchard *et al.*, 1984; Beuchat and Vleck, 1990; Niewiarowski *et al.*, 1992). A linear relationship has been shown in other lizards between the number of neonates and the rate of maternal oxygen consumption, supporting the argument that foetal mass contributes significantly to the observed increase (Birchard *et al.*, 1984; Niewiarowski *et al.*, 1992). This increase can be found in eutherian mammals as well; for example *Plecotus auritus* (long-ear bat), a heterothermic mammal when at rest (not in torpor), also shows a 24 – 25% increase in standard metabolic rate during late pregnancy (Speakman and Racey, 1987).

Table 5.3 Mean mass specific SMR of various lizards

Species	SMR (ml O ₂ g ⁻¹ h ⁻¹)	s.e.	Author
<i>Niveoscincus microlepidotus</i> *			current work
non-pregnant	0.088	0.007	
early-pregnant	0.089	0.005	
mid-pregnant	0.094	0.009	
late-pregnant	0.109	0.004	
<i>Sceloporus jarrovi</i> *			
early-pregnant	0.090	0.003	DeMarco, 1993
mid-pregnant	0.113	0.003	
late-pregnant	0.161	0.003	
<i>Egernia cunninghami</i> *#	0.087	n/a	Bartholomew <i>et al.</i> , 1965 [#]
<i>Tiliqua scincoides</i> *#	0.090	n/a	Wilson 1971, 1974 [#]
<i>Tiliqua rugosa</i> *	0.090	0.005	Christian & Conley, 1994
<i>Physignathus lesuerii</i>	0.080	n/a	Wilson 1971, 1974
<i>Iguana iguana</i>	0.081	n/a	Moberly 1968 [#]
<i>Varanus gouldii</i>	0.077	n/a	Bennett 1972 [#]
<i>Varanus exanthematicus</i>	0.088	0.004	Wood <i>et al.</i> , 1978

* Viviparous species,
not on pregnant animals.
as reported by Wood *et al.*, 1978

The same trend was seen in both resting metabolic measurements and biological elimination studies. However time constraints on this study mandated that the rubidium trials were only conducted during mid-pregnancy. Despite this, the data

showed that there was a significant increase (~29%) in biological elimination of the radioisotope associated with pregnancy. Although the ~18% increase in SMR seen in the mid-pregnant animals was not significant this is may be due to the relatively low number of replicates. Therefore the increased active metabolic rate suggests that resting metabolic rate may have a higher cost earlier in the developmental cycle then demonstrated by this study. This study also suggests that, regardless of any change in SMR, there is a marked increase in active metabolic rate during pregnancy. This increase is probably because the pregnant female is carrying a substantially greater load. Although it has been shown that an increase in weight usually results in a decrease in maximal performance (Shine, 1980), this was not seen in *N. microlepidotus*. However, it is still likely that the increased load will cause pregnant females to “work harder” to maintain performance levels, thereby increasing metabolic costs of mobility in pregnant animals.

The postmortem data showed that no substantial placental transfer of the isotope occurred, suggesting that the biological elimination rates measured were predominately associated with the mother. This suggests that the biological elimination rate is providing a valid measure of the mother’s relative metabolic rate, rather than a combined value resulting from the mother and foetuses. If this reasoning is correct it further suggest that estimates of SMR may also reflect increased maternal metabolism rather than the combined metabolism of the mother and clutch. Few conclusions can be drawn about how much of this increase in SMR directly represents the cost of reproduction to the mother.

The findings presented here suggest that there is a significant metabolic cost associated with pregnancy in *Niveoscincus microlepidotus*. Although it seems that this species is able to minimise other costs associated with reproduction, it is unable to minimise metabolic costs in mid-, and late-pregnancy. It may therefore have to divert resources normally used for growth or other biological processes to the maintenance of a higher metabolic rate. This, combined with the long duration of pregnancy in *N. microlepidotus*, may be the determining factor in the development of a biennial reproductive strategy. A biennial reproductive mode decreases the lifetime fecundity

of a female lizard. This suggests that *N. microlepidotus* invests a significant amount into current reproductive success, rather than being able to reproduce more frequently but presumably less successfully.

The metabolic rates for this study were taken at approximately $25\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$, which was reported to be near to the optimal temperature for non-pregnant *Niveoscincus microlepidotus* (Melville, 1994). However, this study has shown that optimal temperature of pregnant female *N. microlepidotus* is lower than this value. Body temperature is known to affect the metabolic rates of ectothermic animals. Therefore it is a reasonable assumption that the metabolic rate of pregnant females at the modified optimal temperature, may not show the dramatic increase in SMR. Thus this decrease in temperature may reduce the metabolic costs associated with pregnancy. However it is doubtful that a decrease in body temperature of only $2\text{ }^{\circ}\text{C}$ would be able to significantly reduce metabolic rate. It can therefore, be concluded that female *N. microlepidotus* will have a substantial metabolic cost associated with reproduction. However, conclusions about the effects that pregnancy has on SMR or AMR in female *N. microlepidotus* should be made cautiously, with careful consideration about what effect the metabolism of the neonates may be having on total metabolic outcome.

5.6.1 THE USE OF RUBIDIUM⁸⁶

The use of rubidium⁸⁶ is a promising method of examining the active metabolic rates of small animals. The ability to whole body count animals does not place any undue stress on the female, and the radioactivity has shown no observed side effects to either the mother or the neonate. The relatively low emission rate and short half life means that this method may be a suitable for field studies, and is appropriate as a means of investigating the active metabolic rates in many small animals. Although Peters *et al.* (1995) did correlate this method with doubly-labeled water while investigating the AMR of *Dipsosaurus dorsalis*, and found a significant correlation ($r^2 = 0.93$), it has not been correlated in this experiment and therefore can only give a indirect

estimation of metabolic rate. Once correlated,, rubidium⁸⁶ may allow the accurate measuring of active metabolic rate of *Niveoscincus microlepidotus* which until now was impossible to obtain.

CHAPTER 6

SUMMARY AND FINAL DISCUSSION

This thesis provides an analysis of the costs of reproduction in *Niveoscincus microlepidotus*. It attempts to correlate a vast range of potential reproductive costs, including those affecting performance abilities, thermal physiology, thermoregulatory behaviours, and metabolic rates, with reproduction. Potential costs were experimentally investigated in order to achieve a better understanding of the adaptive responses to pregnancy in this alpine skink. This investigation demonstrates how successfully *Niveoscincus microlepidotus* has balanced the costs and benefits of reproduction.

Adaptation of thermoregulatory behaviour appears to play a substantial role in reducing reproductive costs in *Niveoscincus microlepidotus*. Pregnant females of some species select higher body temperatures during gestation (e.g. Werner and Whittaker, 1978; Gibson and Falls, 1979; Greer, 1989; Daut and Andrews, 1993; Blázquez, 1995). However, female *N. microlepidotus* maintain lower body temperatures when pregnant and rarely allow body temperature to exceed 32 °C. In contrast, non-pregnant females were often observed with body temperatures well above 35 °C. This study has also shown that pregnant *N. microlepidotus* select lower maximal setpoints ($T_{\text{move}} \sim 24$ °C) than non-pregnant females ($T_{\text{move}} \sim 28$ °C). It can be assumed that these body temperatures are selected to slow foetal development as long as possible and to increase neonate fitness, by altering timing of parturition which would in turn increase the success of the current reproductive effort.

This study has shown that it may be important for *Niveoscincus microlepidotus* to slow gestation rate so that the embryos reach stage 40 as late in the activity season as possible. It has been demonstrated that the costs to the mother are much higher in the later stages of pregnancy than during any other stage, implying that there may be evolutionary pressure to delay the onset of these high costs until close to hibernation. Swain and Jones (1997) have shown that the closely related *Niveoscincus metallicus*,

supplements yolk reserves through the placenta from stages 35 to late stage 39, but supplementation is at a minimum during stage 40 just prior to birth. In addition it has been observed that in the embryos of *N. microlepidotus*, little yolk remains by the later stages (35 – 39), and none is evident after stage 40 (pers. obs.), even though parturition will not occur for another five or six months. This suggests that either the neonates are utilizing their own fat bodies, or that there is some degree of maternal transfer during the period between exhaustion of the yolk and hibernation. This may support the idea that female *N. microlepidotus* are able to transfer organic nutrients to their embryos in the latter stages of gestation, prior to stage 40, as seen in *N. metallicus*. This would provide a strong evolutionary push to delay the completion of gestation until just prior to the commencement of hibernation, subsequently reducing the length of time that the neonates need to rely on their own fat bodies. This slowing of development until hibernation may minimise many of the costs of carrying a fully developed neonate, while maximising offspring fitness by delaying parturition until the following spring.

Niveoscincus microlepidotus has been shown to have decreased sprinting, climbing, and jumping abilities during late pregnancy at higher temperatures, as well as decreased endurance capacity. Several studies have revealed a decrease in mobility associated with pregnancy in reptile species (Shine, 1980; Bauwens and Thoen, 1981; Smith, 1996; Brodie, 1989; Van Damme *et al.*, 1989; 1990; Cooper *et al.*, 1990; Sinervo *et al.*, 1991). This study suggests that pregnant female *N. microlepidotus* display a physiological shift in their optimal performance temperature from approximately 26 °C for non-pregnant lizards (Melville, 1994) to approximately 24 °C. However, the results suggest that because pregnant females select lower body temperatures, and seldom reach the high temperatures at which performance decreases, pregnant females in the field may not experience a significant drop in performance ability. Thus any decrease in survival as a result of impaired performance may be minimal or non-existent. The decrease in endurance capacity (Kik, 1998; current study), however, is not totally eliminated by the change in optimal performance temperature. This suggests that there may still be a cost of reproduction

associated with mobility, which may result in an increased risk of predation or decreased foraging ability.

Field observations reveal that pregnant female *Niveoscincus microlepidotus* prefer to bask cryptically, thereby selecting safer sites from which they can readily escape without relying on endurance capacity (Kik, 1998). Schwarzkopf and Shine (1992), and Smith (1996) reported that in some species, pregnant females become more sensitive to the distance of an approaching predator, presumably because their ability to escape is impaired. However, this was not observed in *Niveoscincus microlepidotus*; instead locomotory performance is not reduced, and the behavioural modification of the selection of basking sites reduces the need for a high endurance capacity (Kik, 1998). This suggests that a decrease in maximal thermal setpoint (T_{move}) and a drop in endurance capacity may drive thermoregulatory behaviour of pregnant female *Niveoscincus microlepidotus*. This strategy may allow a lower body temperature to be maintained in order to extend the development of the embryos and reduce the risk of predation.

The feeding behaviour of the pregnant females in the laboratory, was observed to be no different to that of non-pregnant females. It was also observed in the field, that pregnant females were consuming both berries (*Cyathodes parifolia*) and various insects that were located within a very small area from the chosen basking site. Although *N. microlepidotus* is not known to be a “sit-and-wait” predator, this predation strategy would comply with observed behaviours and known physiological limitations (Anderson and Karasov, 1981). Therefore, modification of foraging strategy may reduce survival and fecundity costs associated with decreased foraging ability. This suggests that there is little cost of reproduction associated with performance ability in *Niveoscincus microlepidotus* during pregnancy, and what costs are incurred have been minimised by the modification of behaviour and thermal physiology.

This study also investigated the metabolic changes associated with reproduction in *Niveoscincus microlepidotus*. Estimates of metabolism during gestation in viviparous

lizards suggest that standard metabolic rate (SMR) should fit the standard linear relationship between body size and oxygen consumption (DeMarco, 1993). However, the data presented here do not support this assumption. For example, the oxygen consumption of a gravid female *N. microlepidotus* is approximately 24% higher than that of a non-gravid female once corrected for weight, when the linear relationship suggests that there should be no significant differences. It is unclear exactly what accounts for the increase in SMR. It can, however, be assumed that there is an increase due to the maintenance and development needs of the embryos. In viviparous lizard *Sceloporous jarrovi*, the metabolism of the pregnant females is even higher than predicted if metabolism is scaled for embryonic mass (Birchard *et al.*, 1983). This may be accounted for by several factors including an increase in the oxygen consumption of the maternal tissues (Beuchat and Vleck, 1990), increase in gas exchange, and handling of increased nitrogen waste from the embryo (Birchard *et al.*, 1984). However if the assumption of organic matrotrophy is correct then this may be a contributing factor to the large increase in SMR during late pregnancy. This suggests that the substantial increase in oxygen consumption in *N. microlepidotus* during the late stages of pregnancy (35 - 40+) may be in part the result of maternal nutritional input to the young. However, examination of the placental structure of *N. microlepidotus* was beyond the scope of this study, and therefore conclusion about its role in the observed increase in metabolic rates during pregnancy are conjecture at best.

Niveoscincus microlepidotus, displays a physiological optimal performance temperature 26 °C (Melville, 1994). It was this optimal temperature (26 °C) that was used as the basis for the temperature used in the metabolic experiments. Body temperature and metabolic rate are strongly correlated (Wood *et al.*, 1978), suggesting that if the pregnant animals in this study were tested at the modified optimal temperature of 24 °C, there may have been a decrease in metabolic rate. If this assumption holds true, it can be assumed that this physiological shift in optimal performance temperature may decrease the SMR of pregnant females. However, it would be unlikely that the decrease would be sufficient to significantly reduce any metabolic costs of reproduction.

The faster rate of biological elimination of the radionuclide (^{86}Rb) during pregnancy supports the conclusion that there is a significant increase in maternal metabolic rate. This suggests that because the females were not artificially controlled for body temperature and were allowed to select lower body temperatures, the increase in metabolic rate shown is a more accurate picture of the metabolic costs associated with viviparous reproduction. Observed increases may be the result of the same factors as described for SMR, as well as the increased mass of the embryos that the pregnant female has to carry. This implies that a pregnant female must work harder to achieve normal performance levels and thereby incurs an elevated metabolic rate. It was shown that there was an approximate 29% increase in biological elimination rate of the isotope that was predominately the result of increased maternal metabolism. Therefore, if the assumption that most of the increase in estimated active metabolic rate (AMR) was due to maternal metabolism is correct, it may also be assumed that much of the increase in SMR was due to the higher maternal metabolism and not that of the combined maternal and embryonic metabolism. It can be concluded that although pregnant females may have a slightly lower SMR than shown in this study, there is still a substantial metabolic cost associated with reproduction in *Niveoscincus microlepidotus*.

The increase in metabolic rate during pregnancy suggests that a pregnant female may have to consume a larger amount of food than a non-pregnant female in order to maintain physiological functions without relying on fat stores. If a pregnant female is unable to increase food consumption, she will need to reallocate resources from processes such as growth, which is known to have a strong correlation with reproductive fecundity (Shine, 1980). However, it is probable that pregnant females rely heavily on fat bodies, which would have been acquired during the previous non-reproductive summer. This suggests that a female *N. microlepidotus* is able to compensate for the higher resource needs of the pregnant season by utilisation of fat stores acquired during the prior non-pregnant activity season. Thereby lessening the cost of reproduction associated with increased metabolic rate, by actively foraging

more frequently during the previous season when the metabolic cost of movement may be lessened.

Niveoscincus microlepidotus is a true alpine specialist that has a diverse portfolio of mechanisms to minimise the costs of reproduction in a harsh and unstable climate. It has been shown that pregnant *N. microlepidotus* are able to temporarily modify behaviour and thermal physiology to minimise the costs of reproduction. Although, it seems that *N. microlepidotus*, is able to reduce the majority of costs associated with reproduction, the costs are still high enough to require the adoption of a biennial reproductive mode, which in itself represents a high fecundity cost of reproduction. Olsson and Shine (in press) have suggested that this species does not reproduce until its third year, and Hudson (1997) reported that this species lives for approximately 6 years in the field. Subsequently a female *N. microlepidotus* may only be able to produce 2 clutches in her lifetime. This suggests that *N. microlepidotus* may have to invest as much as possible into the current clutch to maximise chances of offspring survival, sacrificing possible future reproductive fecundity by only breeding once every two years.

It can be concluded that *N. microlepidotus* has adopted many behavioural and physiological mechanisms to maximise current reproduction. However the adoption of these mechanisms comes with a substantial cost to future reproduction and this represents a significant reproductive trade-off.

REFERENCES

- Anderson, R. A. and Karasov W. H. 1981 Contrasts in energy intake and expenditure in sit-and-wait and widely foraging lizards. *Oecologia* **49**:67-72
- Andrews, R. M. and Kenney, B. S. 1990. Diel pattern of selected ambient temperature and activity of the sand-swimming lizard *Sphenops sepsoides* (Sauria: Scincidae). *Israel Journal of Zoology* **37**: 65-73.
- Avery, R. 1975. Clutch size and reproductive effort in the lizards: effect of temperature on speed, pauses and gait in *Lacerta vivipara*. *Amphibia-Reptilia* **10**: 77-84.
- Avery, R.A. Bedford, J.D. and Newcombe, C.P. 1982. The role of thermoregulation in lizard biology: predatory efficiency in a temperate diurnal basker. *Behavioural Ecology and Sociobiology*. **11**, 261-267.
- Avery, R. A. and Bond, D.J. 1989. Movement patterns of lacertid lizards: effects of temperature on speed, pause and gait in *Lacerta vivipara*. *Amphibia-Reptilia* **10**: 77-84.
- Beaupre, S. J. 1992. An ecological study of oxygen consumption in the mottled rock rattlesnake, *Crotalus lepidus lepidus*, and the black tail rattle snake *Crotalus molossus molossus*, from two populations. *Physiological Zoology* **66**: 437-454.
- Beaupre, S. J., Dunham, A. E., and Overall, K. L. 1993 Metabolism of a desert lizards: The effects of mass, sex, population of origin, temperature, time of day, and feeding ecology on the oxygen consumption of *Sceloporus merriami*. *Physiological Zoology*. **66**; 128-147.
- Bauwens, D. and Thoen, C. 1981. Escape tactics and vulnerability to predation associated with the reproduction in the lizard (*Lacerta vivipara*). *Journal of Animal Ecology*. **50**: 733 - 743.
- Beuchat, C.A. 1986. Reproductive influences on the thermoregulatory behaviour of a live-bearing lizard. *Copeia*. **4**: 971-979.
- Beuchat, C. A. and Ellner, S. 1987. A quantitative test of life history theory: thermoregulation by a viviparous lizard. *Ecological Monographs*. **57**: 45-60.
- Beuchat, C. A. 1988. Temperature effects during gestation in a viviparous lizard. *Journal of Thermal Biology*. **13**: 135-142.

- Beuchat, C. A. and Vleck, D. 1990. Metabolic rates of female viviparity in a lizard, *Sceloporus jarrovi*. *Physiological Zoology*. **63**: 555-570.
- Birchard, G. F., Black, C. P., Schuett, J. W. and Black, V. 1984. Influence of pregnancy on oxygen consumption, heart rate and haematology in the garter snake: implications for the "cost of reproduction" in live bearing reptiles. *Comparative Biochemistry and Physiology*. **77A**: 519-523.
- Blackburn, D. G., Vitt, L. J. and Beuchat, C. A.. 1984. Eutherian-like reproductive specializations in a viviparous reptile. *Proceedings of National. Academy of Science. U.S.A.* **81**: 4860-4863.
- Blackburn, D. G. 1993. Chorioallantoic placentation in squamata reptiles: Structure, function, development, and evolution. *Journal of Experimental Zoology*. **266**: 414-430.
- Blackburn, D. G. 1994. Standardized criteria for the recognition of embryonic nutritional patterns in squamate reptiles. *Copeia*. **1994**: 925-935.
- Blázquez, M. C. 1995. Body temperature, activity patterns and movements by gravid and non-gravid females of *Malpolon monspessulanus*. *Journal of Herpetology*. **29**: 264-266.
- Brăna, F. 1993. Shifts in body temperature and escape behaviour of female *Podarcis muralis* during pregnancy. *Oikos*. **66**: 216-222.
- Brodie, E. D. 1989. Behavioral modifications as a means of reducing the cost of reproduction. *American Naturalist*. **134**: 225-238.
- Brodie, E.D, Fileti, C.A. Prez, L.E, Ciannoukos, G. Klosterma, L.L., Tsang, P. and McCracken, J. A. (1992) Role of the corpus luteum and progesterone in evolution of vertebrate viviparity. *American Zoologist* **32**: 264-273.
- Carrascal, L. M, López, P., Martín, J. and Salvador, A. 1992. Basking and anti-predator behaviour in a high altitude lizard: Implication of heat-exchange rate. *Ethiology*. **92**: 143-154.
- Christian, K. and Tracy, C. R. 1981. The effect of the thermal environment on the ability of hatchling Galapagos land iguanas to avoid predation during dispersal. *Oecologia*. **49**: 218-223.

- Christian K. and Tracy, C R. 1983. Seasonal shifts in body temperature and use of microhabitats by Galapagos iguanas (*Conolophus pallidus*). *Ecology*. **64**: 463-468.
- Christain, K. A. Baudinette, R. V. and Pamula, Y. 1997. Energetic costs of activity by lizards in the field. *Functional Ecology*. **11**: 392-397.
- Clerke, R. B. and Alford, R. A. 1993. Reproductive biology of four tropical Australian lizards and comments on the factors regulating lizard reproductive cycles. *Journal of Herpetology*. **27**: 400-406.
- Cogger, H. G. 1992. Reptiles and Amphibians of Australia. Reed Books, Sydney.
- Cooper, W. E., Vitt, L. J., Hedges, R. H. and Huey, R. B. 1990. Locomotory impairment and defence in gravid lizards (*Eumeces laticeps*): behavioural shifts in activity may offset costs of reproduction in an active forager. *Behavioural Ecology and Sociobiology*. **27**: 153-157.
- Cooper Jr, W. E. 1997. Factors affecting risk and cost of escape by the broad-headed skink (*Eumeces laticeps*): Predator speed, directness of approach, and female presence. *Herpetologica*. **53**: 464-474.
- Cooper Jr., W. E. 1997. Escape by a refuging prey, the broad-headed skink (*Eumeces laticeps*). *Canadian Journal of Zoology*. **75**: 943-947.
- Cowles, R. B. and Bogert, C. M. 1944. A preliminary study of the thermal requirements of desert reptiles. *Bulletin American Museum. Natural History*. **83**: 265-296.
- Cree, A. and Guillette, L. J. 1995. Biennial reproduction with a fourteen-month pregnancy in the gecko *Hoplodactylus maculatus* from Southern New Zealand. *Journal of Herpetology*. **29**: 163-13.
- Crowley, S. R. and Pietruska, R. D. 1983. Aggressiveness and vocalization in the Leopard lizard *Gambelia wizlizenii*: the influence of temperature. *Animal Behaviour*. **31**: 1055-1060.
- Curio, E 1976. The ethology of predation. Springer-Verlag, New York.
- Daut, E. F. and Andrews, R. M. 1993. The effect of pregnancy on thermoregulatory behavior of the viviparous lizard *Chalcides ocellatus*. *Journal of Herpetology*. **27**, 6-13.

- Dawson, W. R. 1975. On the physiological significance of the preferred body temperatures of reptiles. In *Perspectives of Biophysical Ecology* (ed. D. M. Gates and R. B. Schmerl). *Ecological Studies*. **12**: 443-473. Springer-Verlag, Berlin.
- Department of Environment and Land Management, 1997. Mount Wellington survey
- DeMarco, V. and Guilette, L. J. 1992. Physiological cost of pregnancy in a viviparous lizard (*Sceloporus jarrovi*). *The Journal of Experimental Zoology*. **262**: 383-390.
- DeMarco, V. 1993. Metabolic rates of female viviparous lizards (*Sceloporus jarrovi*) throughout the reproductive cycle: do pregnant lizards adhere to standard allometry. *Physiological Zoology*. **66**: 166-180.
- Doughty, P. and Shine, R. (1997) Detecting life history tradeoffs: Measuring store in "capital" breeders reveals costs of reproduction. *Oecologia*, **110**: 508-513.
- Dufaure, J. P. and Hubert, J. 1961. Table de développement du lézard vivipare: *Lacerta vivipara*. *Jaquin. Arch. Anat. Micr. Morph.* **50**: 309-328.
- Duvall, D., Guillette, L. J. Jr. and Jones, R. E. 1982. Environmental control of reptilian reproductive cycle. In *Biology of the Reptilia* (ed. C. Gans and F. H. Pough). *Physiology* (D.), Academic Press, London. pp. 93-166.
- Fergusson, B., and Bradshaw, S. D., 1991 Plasma arginine vasotocin, progesterone, and luteal development during pregnancy in the viviparous lizards *Tiliqua rugosa*. *General and Comparative Endocrinology*. **82**: 140-151.
- Gallagher, K. J., Morrison, D. A., Shine, R., and Grigg, G. C. 1983 Validation and Use of Na²² turn over to measure food intake in free range lizards. *Oecologia*; **60**; 76-82.
- Garland, T. Jr. 1985. Ontogenetic and individual variation in size, shape and speed in the Australian agamid lizard *Amphibolurus nuchalis*. *Journal of Zoology London*. **207**: 425-439.
- Garrick, L. D. 1974. Reproductive influences on behavioral thermoregulation in the lizard, *Sceloporus cyanogenys*. *Physiology and Behavior*. **12**: 85-91.
- Gibson, R. and Falls, J. B. 1979. Thermal biology of the common garter snake *Thamnophis sirtalis* (L.) I. Temporal variation, environmental effects and sex differences. *Oecologia*. **43**: 79-97.

- Guillette, L. J. 1979. Stimulation of parturition in a viviparous lizard (*Sceloporus jarrovi*) by arginine vasotocin. *General and Comparative Endocrinology*. **38**: 457-460.
- Guillette, L. J., and Jones, R. 1985 Ovarian, oviductal, and placental morphology of the reproductively bimodal lizard, *sceloporus aeneus*. *Journal of Morphology*. **184**: 85-98.
- Guillette, L. J. 1993. The evolution of viviparity in lizards. *Bioscience*. **43**: 742- 751.
- Green, B., Dryden, G., and Dryden, K. 1991 Field energetics of large carnivorous Lizard *Varanus rosenbergi*. *Oecologia*. **88**: 547-551.
- Greer, A. E. 1989. The Biology and Evolution of Australian Lizards. Surrey Beatty & Sons Pty. Ltd. pg117-195.
- Harwood, R. H. 1979. The effect of temperature on the digestive efficiency of three species of lizards, *Cnemidophorus tigris*, *Gerrhonotus multicarinatus* and *Sceloporus occidentalis*. *Comparative Biochemistry and Physiology*. **63A**: 417-433.
- Heatwole, H. 1976. Reptile Ecology. University of Queensland Press. St. Lucia pg 17-40.
- Hertz, P. E., Huey, R. B. and Nevo, E., 1982. Flight versus flight: Body temperature influences defensive responses of lizards. *Animal Behaviour*. **30**: 676-679.
- Hertz, P. E., Huey, R. B. and Nevo, E. 1989. Homage to Santa Anita: Thermal sensitivity of sprint speed in agamid lizards. *Evolution*. **37**: 1075-1084.
- Huey, R. B. and Stevenson, R. D. 1979. Intergrating thermal physiology and ecology of ectotherms: a discussion of approaches. *American Zoology*. **19**: 357-366.
- Huey, R. B., Schneider, W., Erie, G. L. and Stevenson, R. D. 1981. A field-portable racetrack and timer for measuring acceleration and speed of samll cursorial animals. *Experientia*. **37**: 1356-1357.
- Huey, R. B. 1982. Temperature, physiology, and the ecology of reptiles. In Biology of the Reptilia (ed. C. Gans and F.H.Pough),. *Academic Press London*. Vol. **12**: 25-91
- Huey, R. B. and Bennett, A. F. 1987. Phylogenetic studies of coadaptation: Preferred temperatures versus optomal performance temperatures of lizards. *Evolution*. **41**: 1098-1115.

- Hudson, S. (1997) Patterns and life history, growth and morphology in south-eastern Australian viviparous skinks. Ph.D Thesis, La Trobe University, Melbourne, Australia.
- Hutchinson, M. N., Robertson, P. and Rawlinson, P. A. 1989. Redescription and ecology of the endemic Tasmanian scincid lizards *Leiopisma microlepidotum* and *L. pretiosum*. Papers and Proceedings of the Royal Society of Tasmania. **123**: 257-272.
- Hutchinson, M. N., Schwaner, T. D. and Medlock, K. 1988. A new species of scincid lizard (Lygosominae *Leiopisma*) from the highlands of Tasmania. *Proceeding of the Royal Society of Victoria* **100**: 67-73.
- Hutchinson, M. N. and Schwaner, T. D. 1991. Genetic relationships among the Tasmanian scincid lizards of the genus *Niveoscincus*. *Journal of Herpetology* **25**: 49-58.
- Hutchinson, M. N., Donnellan, S. C., Baverstock, P. R., Krieg, M., Simms, S. and Burgin, S. 1990. Immunological relationships and generic revision of the Australian lizards assigned to the genus *Leiopisma* (Scincidae: Lygosominae). *Australian Journal of Zoology*. **38**: 535-540.
- Jones, S. M and Swain, R. 1996. Annual reproductive cycle and annual cycles of reproductive hormones in plasma of female *Niveoscincus metallicus* (Scincidae) from Tasmania. *Journal of Herpetology*. **30**: 140-146.
- Kik, C. 1998 Ecological and behaviour responses associated with pregnancy in the southern snow skink *Niveoscincus microlepidotus*. Unpublished Diplomarbeit der Universitat Ulm.
- Kirkpatrick J.B. 1997 Alpine Tasmania; as illustrated guide to flora and vegetation. Oxford University Press. Melbourne. Pg2-180.
- Lima, S. L. and Dill, L. M. 1990. Behavioral decisions made under the risk of predation: A review and prospectus. *Canadian Journal of Zoology*. **68**: 619-640.
- 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. and Shine, R. 1993. Costs of reproduction in a population of european adders. *Oecologia*. **94**: 488-495.

- Martin, J. and López, P. 1995. Influence of habitat structure on the escape tactics of the lizard *Psammodromus algirus*. *Canadian Journal of Zoology*. **73**: 129-132.
- Mathies, T. and Andrews, R. M. 1997. Influence of pregnancy on the thermal biology of the lizard, *Sceloporus jarrovi*: why do pregnant females exhibit low body temperatures? *Functional Ecology*. **11**: 498-507.
- McCoy, C. J. 1968. Reproductive cycles and viviparity of Guatemalan *Coryophanes pericarinatus* (Reptilia: Iguanidae). *Herpetologica*. **24**: 175-178.
- Melville, J. 1994. Microhabitat separation in two closely related lizards. Honours Thesis. University of Tasmania.
- Melville, J. and Swain, R. 1997a. Spatial separation in two sympatric skinks, *Niveoscincus microlepidotus* and *N. metallicus*, from Tasmania. *Herpetologica*. **53**: 126-132.
- Melville, J. and Swain, R. 1997b. Daily and seasonal activity patterns in two species of high altitude skink, *Niveoscincus microlepidotus* and *N. metallicus*, from Tasmania. *Journal of Herpetology*. **31**: 29-37.
- Melville, J. and Swain, R. 1998a. Evolutionary relationships within the snow skink (Squamata: Scincidae: *Niveoscincus*) of Tasmania. *Australian Biologist*. **11**: 22-25.
- Melville, J. 1998. The Evolution of Locomotory Mode in the Lizard Genus *Niveoscincus*. PhD diss. University of Tasmania.
- Melville, J. and Swain, R. 1999. Home range characteristics in the alpine lizard, *Niveoscincus microlepidotus* (Scincidae) on Mt Wellington, Southern Tasmania, *Wildlife Research*. **26**(3): 263-270.
- Merker, G. P. and Nagy K. A. 1994 Energy utilisation by free range *Sceloporus virgates* lizards. *Ecology*. **65**(2) :575-581.
- Muth, A. 1980. Physiological ecology of desert iguana (*Dipsosaurus dorsalis*) eggs: temperature and water relations. *Ecology*. **61**: 1335-1343.
- Niewiarowski, P. H., and Waldschidt, S.R. (1992). Variation in field metabolic rates of a lizard: use of SMR in ecological contexts. *Functional Ecology*. **6**, 15-22.
- Olsson M. and Shine, R. 1998 Evidence for an adaptive shift in the reproductive cycle of an alpine lizard species. In press

- Patterson, J. W. and Davies, P. M. C. 1978. Preferred body temperature: seasonal and sexual differences in the lizard *Lacerta vivipara*. *Journal of Thermal Biology*. **3**: 39-41.
- Peters E. L., Ibrahim S. A., Tracy, C. R., Whicker, F. W. and Nagy, K. A. 1995, Estimations of the metabolic rate of the desert iguana *Dipsosaurus dorsalis* by radio nucilotide technique. *Physiological Zoology*. **68**(2) ; 316-341
- Pilorge, T. and Barbault, R. 1981. La viviparité chez les lézards: évolution et adaptation. *Acta Oecologica*. **2**: 387-397.
- Reznick, D. 1985. Costs of reproduction: an evualuation of the empirial evidence. *Oikos*. **44**: 257-267.
- Reznick, D. 1992. Measuring the costs of reproduction. *Tree*. **7**: 42-45.
- Saint-Girons, H. and Kramer, E. 1963. Le cycle sexuel chez *Vipera berus* (L.) en montagne. *Revue Suisse de Zoologie* **70**: 199-221.
- Schwarzkopf, L. and Shine, R. 1991. Thermal biology of reproduction in viviparous skinks, *Eulamprus tympanum*: why do gravid females bask more? *Oecologia*. **88**: 562-569.
- Schwarzkopf, L. and Shine, R. 1992. Costs of reproduction in lizards: escape tactics and susceptibility to predation. *Behavioral Ecology and Sociobiology*. **31**: 17-25.
- Schwarzkopf, L., (1996). Decreased food intake in reproducing lizards: A fecundity-dependent cost of reproduction? *Australian Journal of Ecology*. **21**, 355-362.
- Seigel, R. A., Huggins, M. M. and Ford, N. B. 1987. Reduction in locomotor ability as a cost of reproduction in gravid snakes. *Oecologia*. **73**: 481-487.
- Shine, R. 1977. Reproduction in Australian elapid snakes. II. Female reproductive cycles. *Australian Journal of Zoology*. **25**: 655-666.
- Shine, R. 1980. "Costs" of reproduction in reptiles. *Oecologia*. **46**: 92-100.
- Shine, R., (1983) Reptilian reproductive modes: The oviparity- viviparity continuum. *Herpetologica*, **39**, 1-8.
- Shine, R. 1985a. The reproductive biology of Australian reptiles: A search for general patterns. In *Biology of Australasian Frogs and Reptiles* (ed. G. Grigg, R. Shine and H. Ehmann). *Royal Zoological Society of New South Wales*, **1985**, 297-302.

- Shine, R. 1985b. The evolution of viviparity in reptiles: an ecological analysis. In *Biology of the Reptilia* (ed. G. Gans and F. Billett), **16**. Wiley-Interscience, New York.
- Shine, R., (1989). Ecological Influences on the Evolution of Vertebrate Viviparity. Complete Organismal Functions: Intergration and Evolution of Vertebrates. John Wiley and Sons Ltd. ppg 263-277.
- Sinervo, B., Hedges, R. and Adolph, S. C. 1991. Decreased sprint speed as a cost of reproduction in the lizard *Sceloporus occidentalis*: Variation among populations. *Journal of experimental Biology*. **155**: 323-336.
- Smith, G. S. 1996. Correlates of approach distance in the striped lizard (*Sceloporus virgatus*). *Herpetological Journal*. **6**: 56-58.
- Smyth, M and Smith, J. 1968. Oblgatory sperm storage in the skink *Hemiergis peronii*. *Science*. **161**: 575-576.
- Stearns, S. C. 1989. Trade-offs in life-history evolution. *Functional Ecology* **3**: 259-268.
- Speakman, J.R., and Racy, P. R., 1985. Chap 21 The energetics of Pregnancy and lactaton in brown long eared bats *Plecotus auritus*. Recent advancements in the study of bats. Fenton, M.B. Racy P.R. and Raytor, J. Editors. Cambridge University Press. New York. pg. 365-383.
- Stewart, J. R. 1984. Thermal biology of the live bearing lizard *Gerrhonotus coeruleus*. *Herpetologica*. **40**: 349-355.
- Stawart, J.R, and Thompson (1994) Placental structue of the Australian lizards, *Niveoscincus metallicus* (Squamata:scincidae) *Journal of morphology*. **220**: 223-236.
- Swain, R. and Jones, S. 1994. Annual cycle of plasma testosterone and other reproductive parameters in the Tasmanian skink, *Niveoscincus microlepidotus*. *Herpetology*. **50**: 502-509.
- Swain, R. and Jones, S. 1997. Effects of maternal gestational environment on neonate of viviparous lizard, *Niveoscincus metallicus*, partitioning temperature and nutrience. *Abstract of the 3rd world congress of herpitolgy* 203. Ed Rocek, Z. an Hart, S. Prague, CZ.

- Swain, R. and Jones, S. 1997. Maternal-fetal transfer of 3h-labelled leucine in the viviparous lizard, *Niveoscincus metallicus* (Scincidae: Lygosominae). *Journal of Experimental Zoology*. **277**; 139-145.
- Taylor, R. J. 1991. Fauna Conservation in production forests in Tasmania, Forestry Practices Commission unit, Forestry Tasmania.
- Tosini, G. and Avery, R. 1993. Intraspecific variation in lizard thermoregulatory set points: a thermographic study in *Podarcis muralis*. *Journal of Thermal Biology*. **18**: 19-23.
- Van Berkum, F. H., Huey, R. B. and Adams, B. A. 1986. Physiological consequences of thermoregulation in a tropical lizard (*Ameiva festiva*). *Physiological Zoology*. **59**: 464-472.
- Van Damme, R., Bauwens, D. and Verheyen, R. 1989. Effect of relative clutch mass on sprint speed in the lizard *Lacerta vivipara*. *Journal of Herpetology*. **23**: 459-461.
- Van Damme, R., Bauwens, D. and Verheyen, R. F. 1990. Evolutionary rigidity of thermal physiology: the case of the cool temperate lizard *Lacerta vivipara*. *Oikos*. **57**: 61-67.
- Van Damme, R., Bauwens, D., and Verheyen, R. F. 1991. The thermal dependence of feeding behaviour, food consumption and gut-passage time in the lizard *Lacerta vivipara* Jacquin. *Functional Ecology*. **5**: 507-517.
- Wapstra, E. and Swain, R. 1996. Feeding ecology of the Tasmanian Spotted Skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Australian Journal of Zoology*. **44**: 205-213.
- Wapstra, E. 1998. Life History and Reproductive Variation in the Spotted Skink, *Niveoscincus ocellatus*. PhD diss. University of Tasmania, unpublished.
- Weekes, H. C. 1935. A review of placentation among reptiles with particular regard to the function and evolution of the placenta. *Proceedings of the Zool. Society of London*. **1935**: 625-645.
- Wood, S.C., Johansen, K., Glass, M. L., and Malaiya, M. O. 1978. Aerobic metabolism of the *Varanus exanthematicus*; effects of activity, temperature, size. *Journal of Comparative Physiology*. **127**; 331-336.

- Werner, Y. L. and Whittaker, A. H. 1978. Observations and comments on the body temperature of some New Zealand reptiles. *New Zealand Journal of Zoology*. **5**: 376-393.
- Werner, Y. L. 1990. Do gravid females of oviparous gekkonid lizards maintain elevated body temperatures? *Hemidactylus frenatus* and *Lepidodactylus lugubris* on Oahu. *Amphibia-Reptilia*. **11**: 20-204.
- Williams, G. C. 1966. Natural selection, the costs of reproduction, and refinement of Lack's principle. *American Naturalist*. **100**: 687-692.
- Wilson, R.C. and Knowles, D.G. Australian Reptiles. William Collins Pty. Ltd., Sydney.

The Costs of Viviparous Reproduction in Reptiles.

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A review of literature; Submitted to the School of Zoology, University of Tasmania in
partial fulfillment of the requirements for the degree of Bachelor of Science with
Honours. (1998)

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1. Introduction

To be the best in all possible worlds is not biologically possible; to be well adapted to even one world requires compromise. Theoretical studies of life-history evolution identify such compromises as “*tradeoffs*” between life-history variables (Reznick, 1985). Tradeoffs are defined as behavioral or physiological adaptations in which improvements in one characteristic directly relates to the decline in fitness of another related characteristic. Tradeoffs are universal and can be seen in all animals; examples include the turtle’s protective shell and associated loss of mobility, and the mouse eared bat’s echolocation for hunting at night, and the relative lost of sight (Dorit *et al.*, 1991). However, the most frequently encountered examples are associated with the costs of reproduction.

It has been a long standing belief that procreation of the species is the ultimate goal of all life, and therefore an optimal life-history should evolve in order to balance any costs of reproduction with the greatest number of viable offspring. This seemingly simple balance is the driving force behind many of the physiological and behavioral adaptations that many species have adopted. Cost of reproduction can take several forms, but the most common are likely to be higher mortality, reduced energy stores, decreased fecundity, or decreased growth rates (Madsen, 1993, Schwarzkopf, 1996).

This review will focus on the current literature that deals with the costs of reproduction in viviparous lizards, in order to obtain a better understanding of this reproductive mode. Viviparity has evolved in all classes of vertebrates, with the exception of birds and cyclostomes; it is also a relatively uncommon phenomenon within amphibians and teleost fishes (Shine, 1989). However, among the squamate reptiles (lizards and snakes) viviparity has evolved in almost 100 lineages, about 20% of the species (Blackburn, 1982; Guillette, 1993). In fact, of all the viviparous reptiles 71% belong to either two lizard families: Iguanidae and Scincidae, or two families of snakes: Colubridae and Viperidae. These taxa show a discontinuous distribution, which strongly suggests that viviparity is a selective adaptation to external environmental factors. This raises a number of important

questions such as, how and why did viviparity evolve? What physiological and ecological costs are associated with it? How are these costs minimized?

1.1 Terminology

Traditionally there have been three modes of reproduction ascribed to reptiles: 1) *oviparity*, in which eggs are laid and embryos develop outside the mother's body; 2) *ovoviviparity*, in which embryos develop from eggs that are retained within the mother's body but are separated from it by egg membranes; 3) *viviparity*, in which the embryo develops completely within the mother. However, many researchers no longer distinguish between ovoviviparity and viviparity (Tinkle, 1977, Blackburn, 1982, Shine, 1989, and De Fraipont *et al.*, 1996). Despite interspecific differences in the complexity of placentation, all "live bearing" species share the property of retaining "eggs" *in utero* for the entire period of embryonic development so that fully developed young are produced at parturition (Shine, 1983). However, it is still helpful to recognize the two extreme forms of embryonic nutrition that occur: 1) *lecithotrophy*, in which the embryo is supplied nutrients needed for development by the yolk sac; and 2) *matrotrophy or placentotrophy*, in which the embryo is supplied nutrients across the placenta. In both cases, however, gas exchange is facilitated across the placenta (Shine, 1983).

There are at least three hypotheses proposed to explain how viviparity may have evolved (Blackburn, 1995). The first suggests a "gradualistic development" in which successive generations of oviparous lizards retained eggs in their oviducts for incrementally longer periods, until ultimately complete development of the fetuses occurred *in utero*. Thinning of the eggshell and the formation of a simple placenta followed this retention of the egg. A second proposal suggests a process of "saltational evolution" in which females retain their eggs for prolonged periods leading directly to live birth, without any evolutionary intermediate steps or stages. In this scenario, viviparity originated directly from oviparity with little genotypic modification. The final proposal, "punctuated equilibrium" suggests that typical oviparity and viviparity could be a static regent of evolution where prolonged egg retention is a transitory trait only. In this case development could return to oviparity

or evolve to viviparity relatively quickly, depending on the push of external pressures. There is supporting evidence for all of these hypotheses, and in actuality all of these scenarios may have been followed (Blackburn, 1995).

2.0 Evolution of Viviparity

2.1 Selection for Viviparity

Viviparity is defined as the retention of the fetus within the uterus until development is completed at which point fully viable young are born (Shine, 1980; 1989, Schwarzkopf, 1992, Guillette, 1993). So why would such an evolutionary development occur, when the majority of reptiles are oviparous? The Darwinian model of natural selection suggests that any adaptations or traits that would increase the reproductive success of a species should increase in frequency. Therefore there must be some advantage to the increased uterine retention that characterizes viviparity.

Viviparity is only likely to be an advantage if embryonic survival is greater when the embryo is retained *in utero* than when the embryo develops outside the uterus. In the external environment a fetus may face a plethora of hostile factors, such as extremes in temperature, predators, desiccation, and anoxia, and retention within the uterus could minimize some of these factors (Shine, 1983, De Fraipont *et al.*, 1996). One prevailing hypothesis about the evolution of viviparity has been dubbed the “cold climate hypothesis” by Tinkle (1977) and has been supported by others including Blackburn (1982), De Fraipont *et al.*, (1996) and Shine (1983). This hypothesis suggests that viviparity evolved because ectothermic reptiles are dependent on specific temperature ranges for successful development of the fetus. Retention of the embryo permits a greater control of the thermal environment during development, through the mother’s thermoregulatory behavior. In a cold climate, eggs of an oviparous squamate are likely to be laid in situations where temperatures may be below optimal for embryonic development thereby slowing development; this delay could be fatal. In an extreme case, embryonic development may not be completed before ground temperatures reach fatal

lows, killing the unborn clutch (Guillette, 1993). Occupation of a cold climate is the most consistent correlate with viviparity, however, not all cold-climate reptiles are viviparous and not all viviparous lizards inhabit a cold climate. The common occurrence of viviparity in present-day reptilian taxa that do not inhabit cold climates may be due to radiations subsequent to the evolution of this trait (Shine, 1989).

2.2 Physiological Adaptations Necessary for Viviparity

In order for viviparity to be a functional alternative to oviparity several anatomical and physiological changes had to evolve. There are two physiological developments without which there could be no viviparity. The first is the development of a placenta, and the second is an increased duration of egg retention.

The hypothesis suggesting a gradual evolutionary transition between ovoviviparity and viviparity is based on the belief that complete egg retention evolved first, followed later by the loss of the eggshell, and the development of the placenta. If it is accepted that viviparity evolved from oviparity, then a second fact must also be accepted: that the oviparous species ovulate and oviposit eggs which contain all the nutrients required for development (Guillette, 1985). During the evolution of the placenta, a significant decrease in the thickness of the eggshell must have developed. Thinning of the shell wall would result in a reduction in the diffusion distance between the embryonic and maternal blood supplies, allowing the gas and water exchange necessary for the development of the fetus.

Guillette's (1985, 1993) hypothesis suggested that the decrease in shell wall thickness was caused initially by a decrease in the recruitment of the estrogen-dependent uterine glands that are responsible for eggshell development at the onset of the reproductive cycle. This decrease could have been in response to several factors: 1) a reduction in circulating estrogen; 2) an increase in the blocking of the estrogen receptors, or a decrease in the number of the receptors; 3) postreceptor modifications preventing

activities such as transcription and translation; or, 4) a reduction in the number of the stem-cells that are responsible for giving rise to gland cells (Guillette, 1993). The thinning of the eggshell allowed the uterus to be exposed to the chemical signals secreted by the embryo, leading to the maternal recognition of pregnancy, which in turn promoted even further elaboration of the placenta. It is believed that these changes may have been rather rapid due to the fact that the appropriate receptors to allow a response to the endocrine signal of embryonic origin were already present (Guillette, 1993).

However, Blackburn (1982) has argued that, in order for Guillette's (1985, 1993) hypothesis to be correct, it should be possible to find a viviparous specimen that does not have or need a placenta. However, Blackburn's research has shown that in every viviparous squamate that has been examined a placenta is present. Therefore, he believes that the development of the placenta and the thinning of the shell wall must have developed concurrently. He argues that without this concurrent development, there would be no means for gas exchange, or water movement, to occur, both of which are essential for fetal survival. In fact many of the structures or modifications observed might simply be refinements of existing structures that specialized after the evolution of viviparity. An example of this may be the amount of yolk found in the eggs of viviparous species; there is no requirement for a reduction in the yolk but the development of a complex placenta would allow this to occur until complete placentotrophy was achieved (Guillette, 1993).

The other important development necessary for viviparity is prolonged retention of the egg until the fetus has developed completely. Oviparous as well as viviparous reptiles fertilize their eggs internally, and then the shell forms around the fertilized embryo prior to oviposition (Blackburn, 1993). As the evolution from amphibian to reptile occurred the ability to retain the embryo in the uterus developed, providing the initial delay between ovulation and oviposition (Blackburn, 1993). Viviparity requires that the fetus remain in the mother for the entire gestation, not only the short period post ovulation necessary for the shell to form. Although, the mechanisms by which this full term retention are not explicitly understood, recent studies looking at the role of the corpus

luteum and the control of uterine contractions have begun to provide some insight (Fergusson, 1990 & 1992, Callard, 1992, and Blackburn, 1994).

The corpus luteum is a transitory endocrine organ, found in all vertebrate ovaries after ovulation, that secretes primary hormones (Fergusson *et al.*, 1992). In oviparous lizards, there is a positive correlation between the length of activity of the corpus luteum and the duration of egg retention. In viviparous lizards, the corpus luteum releases the primary hormone progesterone, which is present in plasma at relatively high levels until midterm, at which point they start to decline and arginine vasotocin (AVT) and estradiol levels increase (Fergusson *et al.*, 1992). Estradiol and AVT are believed to be directly involved in parturition, by causing the uterine muscles to contract during labor (Callard, 1992). Progesterone is believed to be involved in the inhibition of the contractility of the uterine muscles by inhibiting the secretion of estradiol and AVT, and thus inhibiting parturition. For a more in-depth biochemical discussion see Guillelte (1989; 1993), Fergusson (1990; 1992), Callard (1992), and Blackburn (1994).

3.0 The study of viviparity

The study of reproductive costs involves a complex analysis of the many factors that have an effect on the survival of both the young and of the mother, and relating these factors to the general success of the species. The methodologies designed for achieving these objectives fall into four categories. These are defined as a) a search for phenotypic correlations, b) environmental manipulations, c) a search for genetic correlations, and d) an investigation of potential selective factors (Reznick, 1985).

The first of these (*phenotypic correlations*) seeks to correlate some index of reproductive effort with a potential cost, such as parental survival, parental growth, or future fecundity. Studies in this category deal with naturally occurring variation in reproductive effort. They use statistical correlation between life-history traits to determine costs, which are usually measured as the correlation between some measure of reproductive effort (RE)

and some potential cost to the parent(s) (Reznick, 1985). For example, positive evidence for a cost would be the commonly observed negative correlation between the clutch size and the growth of the gravid female (Madsen *et al.*, 1993).

Experimental manipulations involve the direct manipulation of some aspect of reproduction or some environmental variable that affects reproduction. Experimental manipulations are usually a controlled study in which a single variable is modified, in order to obtain a definitive picture of the effect each variable has on the cost of reproduction (Reznick, 1985). The manipulation of the amount of food available to the female, followed by measurement of the effect of on her reproductive output, is a typical approach using this methodology (e.g. Schwarzkopf *et al.*, 1992).

Genetic correlations are quantitative genetic estimates of the correlation between an index of reproductive effort and some potential cost of reproduction (Reznick, 1985). This type of study depends on the same experimental design as the previous type (*experimental manipulations*); however, correlations are derived from the resemblance or difference between two closely related species. These correlations are interpreted as the measure of the degree to which a single gene affects the expression of two or more traits (Reznick, 1985).

The final methodology (*responses to selection*) involves the use of artificial selection factors to seek correlations between some index of reproductive effort and the potential cost in response (Reznick, 1985). In this type of study, only specific phenotypic traits are allowed to be expressed by the selective breeding of individuals expressing that phenotype. This control of expression yields a population with only selected phenotypes, and thereby allows researchers to measure two or more life-history traits in control and experimental groups, then seek associations between these variables (Bailey, 1992).

These four methods of evaluating the costs of reproduction attempt to gain a better understanding of the tradeoffs associated with reproduction. Baily (1992) suggested that, although these studies examine some of the correlations associated with reproduction,

what is needed is a “simple model” of reproductive costs; only then can a true assessment be obtained. He is supported by Qualls (1997) who suggests that the use of simple indices of reproductive output such as the relative clutch mass (RCM) or energy costs of the clutch is of limited value. Reznick (1985, 1992) believes that evaluation of any cost, such as growth or a decrease in fecundity, cannot be studied using a single life-history variable only. For example, “phenotypic correlation studies” make the assumption that the phenotype is a direct expression of the genotype; however, when the genotype and environment have opposing influences, the phenotype does not necessarily reflect the genetic phenotype but may only reflect the environmental influences (Reznick, 1985, Stearns, 1989). Reznick (1985) and Stearns (1989) argue that only genetic correlations, or response to selection, yield the desired information, because these are the only methods that look at the majority of factors affecting a species. They accept that the other methodologies have merit and suggest that they should not be abandoned, only regarded as being less precise. However, their view is not a universal one others believe that experimental manipulations and phenotypic correlations are essential if a clear picture of the costs of reproduction is ever to be achieved (Shine, 1980, Guillette, 1993, Blackburn, 1994).

4.0 The Costs of Reproduction

All adaptations have costs associated with them and like any other tradeoff situation we can expect a delicate balance between the advantages and the costs of viviparous reproduction. Many of the morphological and behavioral adaptations recognized in viviparous species have been directly attributed to the costs of reproduction (Schwarzkopf *et al.*, 1992). Two main classes of costs have been identified: survival costs, when the effort of the current reproduction yields a probable decrease in an individual's future survival; and fecundity cost, in which the current reproductive investment influences an individual's chance of future successful reproduction (Stearns, 1989, Reznick, 1992). These costs have both physiological and ecological components. Although it is an over simplification to separate ecology from physiology, it may be convenient to do so in order to gain an understanding of the costs of viviparity.

4.1 Predation

Predation is one of the most important forces driving morphology, life-history tactics, and behavior in the natural world (Schwarzkopf *et al.*, 1992). It has been suggested that viviparity may increase the mother's risk of predation (Shine, 1980), and this potential increased risk has caused some researchers to label viviparity as a "risky reproductive method" (Shine, 1980).

Shine suggested that a gravid-female's sprint speed may be significantly lowered when pregnant, thus increasing the predation risk, and therefore resulting in a survival cost of reproduction. His data on six species of skinks showed that there was a 70 – 80% decrease in the sprint speed of females when pregnant (Shine, 1980). In four of these species the average speed declined markedly with higher relative clutch masses (RCM). He suggested that, because the post-partum females were able to run just as quickly as pre-ovulatory subjects, the decrease in sprint speed was probably due to the weight and mass of the clutch, and not due to any decrease in fitness (Shine, 1980). Martin (1996) looked at the effects of rapid changes in mass (due to ingestion of food) on sprint speed

and found very similar results. However, he suggested that sprint speed itself was affected only slightly, and the main cause of the observed decline was a reduction in the acceleration from a stationary start. He concluded, therefore, that it was not “top speed” which was affected, but the time to reach top speed. Martin (1996) also found a significant decrease in endurance, which is also likely to increase the risk of predation. When a lizard is “burdened” with extra weight it may remain in crypsis longer before fleeing (Schwarzkopf, 1992; Martin, 1996), so acceleration and the ability to reach suitable cover could be the keys to successful escape (Martin, 1996).

Schwarzkopf (1992) and Bauwens *et al.* (1981) also counter Shine’s (1980) argument by suggesting that, although they have also observed a decrease in motility associated with pregnancy, gravid females do not show any significant increase in mortality due to predation. They suggest that impaired running speed may not translate into reduced survival, because rarely does the pursuit phase of predation determine the efficiency of the predation. Most of the efficiency of predation is gained or lost in the detection phase. Thus pregnancy may lead to increased predation through an increase in detection risk rather than through a reduction in sprint speed (Schwarzkopf, 1992). Consequently, gravid females may use behavioral modifications to decrease the risk of detection by a potential predator (Qualls, 1997).

4.2 Thermoregulation and Viviparity

Lizards have traditionally been referred to as poikilotherms, indicating that their body temperatures are entirely determined by the ambient temperature. However during activity a lizard’s body temperature can exceed that of “warm blooded” vertebrates. A more helpful classification is to describe lizards as ectotherms, because they rely primarily on an external heat source to maintain a body temperature within a narrow range (Heatwole, 1976). A lizard’s thermal relationship with its environment is, therefore, one of the most important aspects of its life. This relationship determines body temperature, and thus may affect many physiological processes, including reproduction,

metabolism, digestive efficiency, speed of muscular contraction, and the efficiency of neuromuscular coordination (Avery, 1979; Bennett, 1986). Behavioral thermoregulation is a necessary part of an ectotherm's activities, and lizards may spend a significant amount of their active time basking. The need for efficient thermoregulation is likely to be even more important in pregnant lizards because maternal body temperature may affect the gestation time, the sex ratio, the viability, and the size of the young (Avery, 1982; Beuchat, 1988; Schwarzkopf, 1991).

There is a very limited range of optimal incubation temperatures that will allow for proper development of the fetus. This range, however, may not coincide with the mother's optimal temperatures for various physiological functions (Beuchat, 1987). Consequently, a pregnant female may need to maintain a body temperature that is a compromise between her own thermal optima, and those necessary for her embryos' development (Beuchat, 1987; Schwarzkopf *et al.*, 1992). This compromise is critical, because if the temperature is either too high or too low it can result in reduced viability or increase abnormalities of the young (Beuchat, 1987). For example, in *Sceloporus jarrovi*, gestation at the optimal body temperature of 32 (C, resulted in an average of 95% viability, with no obvious morphological or behavioral abnormalities in the offspring. However, an increase or decrease of as little as 4 (C led to over 50% mortality or congenital birth defects (Beuchat, 1987).

It has been suggested that the temperatures experienced by the fetus can accelerate or retard its rate of development (Shine, 1980; Avery, 1982; Beuchat, 1987; Schwarzkopf *et al.*, 1991 & 1992). However, rapid development is not necessarily better, because the duration of embryonic development cannot be changed without significantly affecting the fitness of the offspring. Schwarzkopf *et al.* (1991) showed that when given the opportunity gravid females, in the laboratory, chose to maintain their body temperature at field values, thereby keeping the same development rate, and optimizing clutch viability and health.

The increased mass of a female due to her clutch may decrease her heating rate, requiring her to devote a larger proportion of time to thermoregulating, in order to maintain an optimal body temperature (Shine, 1980; Avery, 1982; Schwarzkopf *et al.*, 1992). A disproportional amount of time devoted to thermoregulation could affect the fitness of the female by increasing the risk of predation, and by reducing possible future fecundity (Shine, 1980; Madsen, 1987). Shine (1980) suggested that, because gravid females spend an increased amount of time in the open thermoregulating, they have increased exposure to diurnal predators, especially avian and other predators that rely on sight to locate prey. Although this is an intuitive assumption, other studies have not shown a correlation between increased basking time and predation, and have suggested that gravid lizards may have adopted behavioral means to reduce risk (Bauwens, 1981; Schwarzkopf *et al.*, 1992). Schwarzkopf *et al.* (1992) showed, in a series of predation trials using skinks (*Eulamprus tympanum*), that pregnant females were never taken more frequently than non-gravid females or males.

In some species of viviparous reptiles it is necessary to change maternal body temperature to best suit the developmental needs of the fetuses. It has been shown that this can either be a decrease or an increase in average temperature. For example, several species of reptiles, including *Lacerta vivipara*, *Sceloporus cyanogenys* and *Eulamprus tympanum*, decreased body temperature during pregnancy, presumably to optimize development of the fetuses (Garrick, 1974; Patterson *et al.*, 1978, -from table 2 by Shine, 1980; Shwarzkopf *et al.*, 1991). In contrast, an increase in body temperature is seen in several other viviparous species, such as *Crotalus viridis*, *Thamnophis ordinoides*, and *Hoplodactylus maculatus* (Hirth *et al.*, 1969; Gibson *et al.*, 1979; Werner *et al.*, 1978, -from table2 by Shine, 1980).

The modification of average maternal body temperature during pregnancy may cause the gravid females to sacrifice future fecundity for current reproductive effort (Schwarzkopf, 1991; Beuchat, 1988; Avery, 1982). For example, she may be unable to apportion sufficient energy to growth, and it has been suggested that lifetime fecundity in lizards is directly dependent on body size (Schwarzkopf, 1991; Beuchat, 1988; Avery, 1982).

Nevertheless any costs associated with prolonged gestation might be minimized if gestation time were reduced. However, the potential costs associated with the increased basking necessary to accelerate gestation, such as an increased risk of predation, make it difficult to predict the better solution (Shine, 1980; Schwarzkopf *et al.*, 1991). As well as the possibility of decreased viability of the young, arising from accelerated gestation, further complicates the issue (Shine, 1980; Schwarzkopf *et al.*, 1991).

4.3 Resource Allocation

Resource allocation involves selectively apportioning necessary resources to one biological process while compromising the amounts that another receives. There are two ways in which an individual can reallocate resources: physiologically, in which a morphological structure or method of substrate utilization is modified in order to increase survival; and behaviorally, where an individual modifies its behavior to best suit the changes in resource requirements and availability (Beuchat *et al.*, 1989).

4.3.1 Metabolism during Pregnancy.

Metabolism is the sum total of all biochemical processes that occur within an organism that deal with its energy and substrate requirements. Metabolism is important to understanding many aspects of physiological ecology, including microhabitat selection, patterns of life-histories, and population dynamics (Beaupre, 1992; Beuchat *et al.*, 1989).

The metabolic rate of a reptile is sensitive to many factors, including the mass of the animal, body temperature, diel cycles, sex, feeding state, and reproductive state (Beaupre, 1992; Beaupre *et al.*, 1992). Maintenance of a standard metabolic rate (SMR; *the lowest metabolic rate of an ectotherm, when at rest, post thermoregulatory, and undisturbed at a given temperature*) is critical to the survival of the individual (Wood *et al.*, 1978; Niewiarowski *et al.*, 1992). Variations in metabolic requirements will greatly affect the

resources available for growth, reproduction, energy storage (lipids), and activity (Beaupre *et al.*, 1992).

In mammals there is an increasing demand on the mother's metabolic rate as the fetus grows and parturition approaches. This increased demand may be due to the costs associated with the placenta, and uterus, gas exchange, and the passing of nutrients to the fetus (Beaupre, 1992; Beaupre *et al.*, 1992; Beuchat *et al.*, 1989). Viviparous lizards are predominately lecithotrophic and in most cases females have only to supply the embryos with water and gas exchange. Costs associated with nutrient transfer are consequently minimal or non-existent (DeMarco *et al.*, 1992). It has been reported that, in some viviparous lizards and snakes, the total oxygen consumption may be greater than the sum of the maternal somatic metabolism plus that of the clutch (DeMarco *et al.*, 1992). Beuchat *et al.* (1989) showed that oxygen consumption of the gravid female increases as the embryos develop. For example, in the viviparous lizard *Sceloporus jarrovi*, the oxygen consumption of a pregnant female is greater than that of males, of the same weight, when corrected for temperature and the oxygen consumption of the clutch (Birchard *et al.*, 1983).

Past estimates of oxygen consumption have assumed that the metabolic rate of gravid lizards can be matched to the standard allometric relationship between metabolism and body mass (Wood, 1978; Beuchat, 1989). However, others have suggested that the increase in oxygen consumption may not fit this allometric relationship, and may result from a combination of the fetal mass, maternal physiological changes, and maternal body mass (Bickler, 1985; Birchard *et al.*, 1983). Several studies have further suggested that the increase in facilitated gas exchange and nitrogen waste handling resulting from embryonic metabolism has an effect on the resting heart rate of the gravid female, thereby increasing metabolic demand (Bickler, 1985; Birchard *et al.*, 1983; Niewiarowski *et al.*, 1992). In pregnant female garter snakes, *Thamnophis sirtalis*, an increase in mean heart rate of 40% to 130% was shown by Birchard *et al.* (1984). A linear relationship has been found between the number of neonates and the rate of maternal oxygen consumption,

lending weight to the argument that fetal mass also contributes significantly to the observed increase (Birchard *et al.*, 1983; Niewiarowski *et al.*, 1992).

4.3.2 Growth during Pregnancy

It is crucial for a gravid female to adjust her energy budget to encompass the increased energy demands of the fetuses, because any debt could lead to death, weakness, and loss of the fetuses (Madsen *et al.*, 1993). However, it may simply be that the gravid females may not have enough energy to devote to growth, which could result in a reduction in future fecundity (Beaupre, 1992).

Ectothermic reptiles in temperate climates have a finite activity period in which to accomplish all necessary life functions, including foraging, hunting, thermoregulation, and mating. It has been suggested that gravid females must spend an increased amount of time thermoregulating in order to maintain a thermal optimum (Shine, 1980). However, if a female spends too much of its active period thermoregulating she will not have sufficient time for other necessities such as foraging. This has been shown to occur in some skinks and a complete cessation of feeding during pregnancy has been shown in some snakes (Schwarzkopf, 1996; Shine, 1988). Examples of viviparous lizards that decrease food intake during pregnancy include *Egernia striolata* and *Leiolopisma otagoense*, and examples among snakes includes *Notechis scutatus* and *Pseudoechis porphyriacus* (Schwarzkopf, 1996; Shine, 1980). A reduction or cessation in food intake would require the female to draw upon stored energy reserves (fat stores) during pregnancy and may, consequently, have an important implications for the future fecundity (Schwarzkopf, 1996). This may be due to the increased demand of the metabolic costs.

5.0 Behavioral Modifications

Part of a successful life-history is the ability to make tradeoffs necessary to ensure minimization of costs. These tradeoffs are not dependent on any single mechanism but consist of multiple behavioral concessions (Brodie, 1988).

Although it seems intuitive that slower sprint speeds and increased time basking would increase the risk of predation, detailed studies have not always supported these ideas (Madsen, 1987). There seems to be evidence that behavioral modifications have been developed to reduce the risk of predation. Schwarzkopf and Shine (1992) described the escape tactics and pregnancy in skinks. Their study suggested that successful predation may be divided into, *detection, pursuit, subjugation, and consumption*, and an unsuccessful predation attempt may be due to inefficiency in any one of these four phases. There is probably little variation, on the successful predation of lizards, in the efficiency of the subjugation and consumption phases because many lizards have no special mechanisms to facilitate escape once caught, with the exception of autotomy (Schwarzkopf *et al.*, 1992). Instead they utilize the tactics of *flight, crypsis*, or “*known escape routes*”, all of which are concerned with avoidance of detection or to facilitate escape (Schwarzkopf *et al.*, 1992).

Shine's (1980) hypothesis, suggesting that a decrease in sprint speed and endurance can lead to increased predation, assumes that the majority of predatory efficiency occurs in the pursuit phase. However, most lizards would not be able to outrun an attack from an avian predator using speed or endurance alone, so necessity for substantial sustained speed or endurance is questionable (Schwarzkopf, 1992).

Nevertheless, it is generally accepted that there are significant changes in the gravid female's behavior that may help to dramatically reduce the risk of predation (Shine 1980, 1989; Resnick, 1985; Schwarzkopf, 1992). These modifications include a decrease in home range size, use of shorter known escape routes, increased crypsis, and increased wariness (Schwarzkopf, 1992). Schwarzkopf's (1992) work with *Eulamprus tympanum*

suggested that flight may be the last resort for many gravid females. Her study showed that gravid females generally allowed a predator to come much closer than did non-gravid females and males. The gravid female would typically freeze (crypsis) after noticing the potential predator and would remain this way until the predator moved into a predetermined approach distance, at which point she would suddenly sprint away, using a known escape route (Schwarzkopf, 1992; Bauwens, 1981). The distance ran to reach cover was not significantly shorter than the distance for non gravid females, but the flight distance was directly correlated with minimum approach distance (Schwarzkopf, 1992).

Further adaptations shown by some species of viviparous lizards may be to delay reproduction until later in their life cycle. For example, the alpine skink, *Niveoscincus mircolepidotus*, does not breed until about age three allowing the female sufficient time to grow and prepare for the costs of pregnancy. This adaptation increases the chances of a successful current reproduction while minimizing the risk to the female's fitness (Olsson *et al.*, unpub). However, females only breed biennially or triennially, thereby allowing the female to recover from any negative costs to its fitness as a result of pregnancy (Swain, 1972; Hutchinson *et al.*, 1989; Olsson, *et al.*, in). Although this species may only live about 6 years, it risks lifetime fecundity to maximize the chance of current reproductive success. (Hudson, 1997)

Finally, a few species have an unusual reproductive pattern in which the female will delay parturition until after the end of the hibernation period (over winter) even though their embryos are completely developed prior to hibernation. It is believed that this has evolved to increase the chance that the offspring will survive, by giving the juveniles the majority of the spring, summer, autumn months to grow and prepare for winter (Cree *et al.*, 1995; Olsson *et al.*, in press).

6.0 Conclusions

When a beneficial adaptation in one trait is associated with a detrimental change in another it represents a tradeoff in life-history. Such tradeoffs represent the currency paid

for minimizing costs (Stearns, 1989). Reproduction has been associated with the most prominent costs. These costs in viviparous reproduction in reptiles can take many forms including death, decreased growth, increased predation, and decreased future fecundity.

Lizards have evolved viviparity in order to balance the costs and advantages of reproduction. These costs have elicited much interest in the physiological ecology field many details about the nature and prevalence of the costs have yet to be resolved, and there is still much debate about what costs affect the life history of reptiles. Although the controversy endures, the continued study will remain a necessary part of gaining insight into the evolution of successful life-history strategies for many years.

References

- Andrews, R. M. and Pough, H. (1985) Metabolism of squamate reptiles: allometric and ecological relationships. *Physiological Zoology* **58**:214-231.
- Avery, R.A. Bedford, J.D. and Newcombe, C.P. (1982). The role of thermoregulation in lizard biology: predatory efficiency in a temperate diurnal basker. *Behavioural Ecology and Sociobiology*. **11**:261-267.
- Avery, R.A. (1970). Utilization of caudal fat by hibernating common lizards *Lacerta vivipara*. *Comparative Biochemistry and Physiology*. **37**, 119-121.
- Bailey, R. C. (1992). Why we should stop trying to measure the cost of reproduction correctly. *Oikos*, **65**, 349- 352.
- Bauwens, D. and Thoen, C. (1981). Escape tactics and vulnerability to predation associated with reproduction in the lizard *Lacerta Vivipara*. *Journal of Animal Ecology*. **50**: 733-743.
- Beaupre, S. J. (1993). An ecological study of oxygen consumption in the mottled rock rattlesnake, *crotalus lepidus lepidus*, and the black-tailed rattle snake, *crotalus Molossus molossus*, from Two Populations. *Physiological Zoology*. **66**, 437- 454.
- Beaupre, S. J. Dunham, A. E. and Overall, K. L. (1993). Metabolism of a desert lizard: The effects of mass, sex, population of origin, temperature, time of day, and feeding on oxygen consumption of *Sceloporus merriami*. *Physiological Zoology*. **66**, 128-147.
- Beuchat, C. A. (1988). Temperature effects during gestation in a viviparous lizard. *J. Thermal Biology*. **13**, 135-142.
- Beuchat, C. A. and Vleck D. (1990). Metabolic consequences of viviparity in a lizard, *Sceloporus Jarrovi*. *Physiological Zoology*. **63**, 555-570.
- Birchard, G. F. Black C. P. Schuett, G. W. and Black, V. (1984). Influence of pregnancy on oxygen consumption, heart rate, and hematology in the garter snake: Implications for the costs of reproduction in live bearing reptiles. *Comparative Biochemistry and Physiology* **77**, 519-523.
- Blackburn, D. G. (1982). Evolutionary origins of viviparity in the reptilia. I. Sauria. *Amphibia- Reptilia*. **3**, 185-205.
- Blackburn, D. G. (1992). Reproduction in viviparous south American lizards of the genus *mabuya*. 150-162 *Reproductive Biology of South American Vertebrates*, Hamlett press.

- Blackburn, D. G. (1993). Chorioallantoic placentation in squamate reptiles: Structure, function, development, and evolution. *Journal of Experimental Zoology*. **266**, 414-430.
- Blackburn, D. G. (1994). Standardized criteria for the recognition of embryonic nutritional patterns in squamate reptiles. *Copeia* **1994**, 925-935.
- Blackburn, D.G (1995). Saltationist and punctuated equilibrium models for the evolution of viviparity and placentation. *Journal of Theoretical Biology*. **174**, 199-216.
- Brodie, E. D. (1989). Behavioral modifications as a means of reducing the cost of reproduction. *American Naturalist*. **134**, 225-238.
- Callard, I. P. Fileti, L.A. Perez, L.E. Giannoukos, G. Klosterman, L.L. Tsang, P. and McCracken, J.A., (1992). Role of the corpus luteum and progesterone in the evolution of vertebrate viviparity. *American Zoology*. **32**, 264-275.
- Callard, I. P. and Kleis, S. M. (1992). Reproduction in reptiles. fundamentals of comparative vertebrate endocrinology. Boston University Press pp. 187-197.
- Christain, K. A. Baudinette, R. V. and Pamula, Y. (1997). Energetic costs of activity by lizards in the field. *Functional Ecology*. **11**, 392-397.
- Caswell, H. (1982) Optimal life histories and age-specific costs of reproduction. *Journal of Theoretical Biology*. **98**, 519-529.
- De Fraipont, M. Clobert, J. Barbault, R. (1996). Evolution of oviparity with egg guarding and viviparity in lizards and snakes: A phylogenetic analysis. *Evolution* **50**, 391-400.
- DeMarco, V. and Guillette, L. J., (1992). Physiological Cost of Pregnancy in a Viviparous Lizard (*Sceloporous jarrovi*) *Journal of Experimental Zoology*. **262**, 383-390
- DeMarco, V. (1993). Metabolic rates of female viviparous lizards (*Sceloporous jarrovi*) throughout the reproductive cycle: Do pregnant lizards adhere to standard allometry? *Physiological Zoology*. **66**, 166-180.
- Doughty, P. and Shine, R. (1997) Detecting life history tradeoffs: Measuring store in "capital" breeders reveals costs of reproduction. *Oecologia*, **110**, 508-513.
- Dorit, R.L. Walker, W.F. and Barns, R. D. (1991). *Zoology international ed.* Saunder College Pub. pp 212 & 380.

- Fergusson, B. and Bradshaw, S. D. (1991) Plasma arginine vasotcin, progesterone, and luteal development during pregnancy in the viviparous lizard *Tiliqua rugosa*. *General and Comparative Endocrinology*. **82**, 140-151.
- Fergusson, B. and Bradshaw, S. D. (1991) In Vitro uterin contractions in the viviparous lizard *tiligua rugosa*: Effects of gestation and steroid pretreatment in vivo. *General and Comparative Endocrinology* **86**, 203-210.
- Garrick, L.D., (1974) Reproductive influences on behavioral thermoregulation in the lizard *Sceloporus cyanogenys*. *Physiological Behaviour*. **12**, 85-91.
- Gibson, P.T., and Falls. J.B. (1979). Thermal biology of the common garter snake *thamnophis sirtalis*: I. Temporal variations, environmental effects and sex differences. *Oecologia*, **43**, 79-97.
- Guillette, L. J., and Jones, R. (1985) Ovarian, oviductal, and placental morphology of the reproductively bimodal lizard, *sceloporus aeneus*. *Journal of Morphology*. **184**: 85-98.
- Guillette, L. J. (1993). The evolution of viviparity in lizards. *Bioscience*, **43**, 742- 751.
- Heatwole, H. (1976) *Reptile Ecology*. University of Queensland Press, St Lucia.
- Hirth, H. F., and King, A.C., (1969). Body Temperature of Snake in difference seasons. *Journal of Herpatology*. **3**, 101-102.
- Hudson, S. (1997) Patterns and life history, growth and morphology in south-eastern Australian viviparous skinks. Ph.D Thesis, La Trobe University, Melbroure, Australia.
- James, C. and Shine R. (1985). The seasonal timing of reproduction: a tropical-temperate comparison in Australian lizards. *Oecologia*, **67**, 464-474.
- Koufopanou, V. and Bell, G. (1984). Measuring the cost of reproduction: IV predation experiments with *daphnia pulex*. *Oecologia*, **64**, 81-86.
- Leal, M. and Rodriguez-Robles, J. A. (1995). Anti-predator responses of *anolis critstellus* (sauria: *Polychrotadae*) *Copeia*, **1995**,: 155-161.
- Luiselli, L. Capula, M. and Shine, R. (1996). Reproductive output, costs of reproduction, and ecology of the smooth snake, *Coronella austriaca*, in the eastern Italian Alps. *Oecologia* **106**, 100-110.
- Madsen, T. and Shine R., (1993). Costs of reproduction in a population of European adders. *Oecologia*, **94**, 488-495.

- Martin, J. and Lopez, P. (1995). Influence of Habitat Structure on the Escape Tactics of the lizard *Psammodromus algirus*. *Canadian Journal of Zoology*. **73**, 129-132.
- Martin, J. (1996). The effects of recent feeding on locomotion performance of juvenile *Psammodromus algirus* lizards. *Functional Ecology*. **10**, 390-395.
- Melville, J. and Swain R., (1998). Home ranges characteristics of an alpine lizard, *Niveoscincus micrlepidotus* (Scincidae), on Mt Wellington, southern Tasmania. In Press.
- Niewiarowski, P. H., and Waldschidt, S.R. (1992). Variation in field metabolic rates of a lizard: use of SMR in ecological contexts. *Functional Ecology*. **6**, 15-22.
- Niewiarowski, P. H., and Dunham, A. E. (1994). The evolution of reproductive effort in squamate reptiles: Costs, tradeoffs, and assumptions reconsidered. *Evolution*. **48**: 137-145.
- Patterson, J.W., and Davies, P.M.C., (1978). Preferred body temperatures: Seasonal and sexual differences in the lizard *Lacerta vivipara*. *Journal Thermal Biology* **3**, 39-49.
- Pough, F.H., and Andrews R., (1985). Use of anaerobic metabolism by free ranging lizards. *Physiology Zoology*. **58**, 205-213.
- Qualls, F.J., and Shine, R. (1997). Geographic variation in costs of reproduction in the scincid lizard *lampropholis guichenoti*. *Functional Ecology*. **11**, 757-763.
- Rismiller, P.D., and Heldmaier, G., (1991). Seasonal changes in daily metabolic patterns of *Lacerta viridis*. *Journal of Comparative Physiology Biology*. **161**, 482-488.
- Resnick, D., Sexton, O.J., and Mantis, C., (1981). Initial prey preferences in the lizard *Sceloporus malachiticus*. *Copia*, **3**, 681-686.
- Resnick, D., (1992). Measuring the costs of reproduction. *Tree*, **2**, 42-45.
- Renick D., (1985). Costs of reproduction; an evaluation of the empirical evidence. *Oikos*, **44**, 257-267.
- Schwarzkopf, L., and Shine R., (1991). Thermal biology of reproduction in viviparous skinks, *Eulamprus tympanum*: Why do gravid females bask more? *Oecologia*, **88**, 562-569.
- Schwarzkopf, L. and Shine R., (1992). Cost of reproduction in lizards: escape tactics and susceptibility to predation. *Behavioural Ecology Sociobiology*. **31**, 17-25.

- Schwarzkopf, L., (1996). Decreased food intake in reproducing lizards: A fecundity-dependent cost of reproduction? *Australian Journal of Ecology*. **21**, 355-362.
- Shine, R., (1980). Costs of reproduction in reptiles. *Oecologia*, **46**, 92-100.
- Shine, R., (1983) Reptilian reproductive modes: The oviparity- viviparity continuum. *Herpetologica*, **39**, 1-8.
- Shine, R., (1989). Ecological Influences on the Evolution of Vertebrate Viviparity. Complete Organismal Functions: Intergration and Evolution of Vertabrates. John Wiley and Sons Ltd. ppg 263-277.
- Sinervo, B., and DeNardo, D. F. (1996). Costs of Reproduction in the Wild: Path Analysis of Natural Selection and Experimental Tests of Causation. *Evolution*, **50**, 1299-1313.
- Sorci, G., Colbert, J., Michalakakis, Y., (1996). Cost of reproduction and cost of parasitism in the common lizard, *Lacerta vivipara*. *Oikos*, **76**, 121-130.
- Stearns, S.C. (1980). A new view of life- history evolution. *Oikos*, **35**, 266-281.
- Stearns, S.C. (1989). Trade-offs in life history evolution. *Functional Ecology*. **3**, 259-268.
- Stewart, J. R. and Blackburn, D.G. (1988). Reptilian placentation: structural diversity and terminology. *Copeia*, **1988**, 839-852.
- Tinkle, D.W. and Gibbson J.W. (1977). The distribution and evolution of viviparity in reptiles. *Museum Zoology University Michigan*. **154**: 1-55.
- Wood, S.C., Johanson, K., Glass, M. L., and Maloiy G.M.O. (1978). Aerobic metabolism of the lizard *Varanus exanthematicus*: effects of activity, temperature, and size. *Journal of Comparative Physiology*. **127**, 331-336.