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Parental effects in two species of viviparous lizards: *Niveoscincus microlepidotus* and *N. ocellatus*



by

Natalia Atkins BSc. (Hons)

Submitted in fulfillment of the requirements for the Degree of
Doctor of Philosophy, School of Zoology, University of
Tasmania
(June, 2007)

Declaration

This thesis contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution, and to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due acknowledgement is made in the text of the thesis.

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This thesis contains the following published papers:

Chapter 2: **Atkins, N, Swain R, Jones SM. 2007a.** Are babies better in autumn or spring? The consequences of extending gestation in a biennially reproducing viviparous lizard. *Journal of Experimental Zoology* **307A**: 397-405.

Chapter 3: **Atkins N, Swain R, Jones SM. 2006.** Does date of birth or a capacity for facultative placentotrophy influence offspring quality in a viviparous skink, *Niveoscincus microlepidotus*? *Australian Journal of Zoology* **54**: 369-374.

Chapter 4: **Atkins N, Swain R, Wapstra E, Jones SM. 2007b.** Late stage deferral of parturition in the viviparous lizard *Niveoscincus ocellatus* (Gray 1845): implication for offspring quality and survival. *Biological Journal of the Linnean Society* **90**: 735-746.

Chapter 5: **Atkins N, Jones SM, Guillette Jr. LJ. 2006.** Timing of parturition in two species of viviparous lizard: influences of beta-adrenergic stimulation and temperature upon uterine responses to arginine vasotocin (AVT). *Journal of Comparative Physiology B* **176**: 783-792.

Appendix 1: **Atkins N, Wapstra E. 2004.** Successful treatment of a mite infestation in gravid spotted snow skinks (*Niveoscincus ocellatus*). *Herpetofauna* **34**: 66-69.

Abstract

This thesis focuses on maternal contributions to offspring fitness in viviparous lizards. Although parental effects may include both pre- and postpartum components, the majority of squamate reptile species exhibit no parental care: parental effects on offspring fitness can, therefore, be imposed only until the time of oviposition or parturition.

In viviparous reptiles, offspring are retained *in utero* for the entire duration of embryogenesis, but in most species the majority of embryonic nutrition is supplied through the yolk with a small contribution by a simple placenta. In some reptilian species, viviparity has evolved further, resulting in a diverse range of placental arrangements and a complete spectrum of embryonic nutritional modes being displayed across a wide range of taxa. It has been suggested that facultative placentotrophy (the ability to supplement an adequate yolk supply) allows the introduction of flexibility into the timing of parturition by providing embryos with additional energy stores to utilise if parturition is delayed. My study species were two closely related viviparous lizards found in Tasmania, Australia. Previously, embryonic nutrition has been shown to be predominantly placentotrophic in *Niveoscincus ocellatus*; I have now determined that embryonic nutrition is predominantly lecithotrophic in *N. microlepidotus*, and that females may utilise facultative placentotrophy only in some years.

My thesis investigated the major hypothesis that deferral of parturition after completion of embryonic development is a key strategy employed by females of viviparous lizards to maximise offspring fitness. The three interlinked papers on this theme that I have included in my thesis support my hypothesis. In *N. ocellatus*, deferring parturition in response to cold conditions had no effect on offspring

phenotype at birth, dispersal distance or survivorship of offspring after release; however, there was a significant negative effect on offspring growth measured after release, which has profound implications for age and size at maturity. I found that females from a high elevation population were less able to defer birth under “long” periods (three weeks) of cold conditions than females of a low elevation population. I attribute the reduced ability of females from the high elevation population to defer parturition to selection for preventing births too close to winter.

However, in the biennially reproducing *N. microlepidotus*, my results have identified that the naturally protracted deferral of parturition from autumn until spring represents a trade-off between offspring quality and offspring size. Finer scale variation in the timing of parturition also influences neonatal characteristics: I have shown that there is an effect of date of birth on several key offspring characteristics at birth in *N. microlepidotus*. Experimental manipulations of the maternal environment demonstrated that females are able to defer birth for an additional four weeks at the end of gestation, but with no significant effect on offspring characteristics.

How is the timing of parturition determined if fully developed embryos may be held *in utero* for significant periods of time? I discovered that in *N. microlepidotus* the uteri are equally responsive to hormonal stimulation (arginine vasotocin (AVT) and prostaglandin ($\text{PGF}_{2\alpha}$) in autumn and spring. In both *N. microlepidotus* and *N. ocellatus*, females are more responsive to AVT than to $\text{PGF}_{2\alpha}$, and the response to AVT is decreased, but not prevented, by β -adrenergic stimulation. In *N. ocellatus*, temperature modulates the response to AVT *in vivo*, with the time to parturition increasing as temperature decreases. In these viviparous

species, then, the endocrine cascade leading to parturition is modulated by the β -adrenergic system.

The final component of the thesis investigated male reproductive success in a population of *N. ocellatus*. I determined the paternity of 65% of the offspring: the results demonstrate that the species has a high level (93%) of multiple paternity within litters, with females having access to many males. While female size is correlated with litter size, I was unable to identify any factors that determine male reproductive success. In addition, the size of the father within a litter had no effect on offspring characteristics at birth, and no measured parental characteristics were determinants of offspring survival.

This thesis has demonstrated that females of viviparous lizards exhibit a suite of characteristics that enable them to manipulate offspring characteristics through the control of the timing of parturition. This provides new evidence to support Shine's "Maternal Manipulation Hypothesis".

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Chapter 1

General Introduction

Phenotypic variation among offspring is attributable not only to genotypic variation but also to the environmental experience of the parents. Such variation is termed parental effects, with maternal effects usually being more significant (Mousseau & Fox, 1998; Qvarnstrom & Price, 2001; Reinhold, 2002). Parental effects tend to be more important early in life (Lindholm, Hunt & Brooks, 2006; Reinhold, 2002) by, for example, affecting juvenile dispersal (Massot & Clobert, 2000; Sinervo *et al.*, 2006); however, parental effects may be significant and persist into adult life (Mousseau & Fox, 1998). Maternal immune experience has substantial long-term effects on offspring antibody responses that persist in fully grown free-living song sparrow (*Melospiza melodia*) offspring (Reid *et al.*, 2006). As another example of a persistent maternal effect, male attractiveness to oestrous female house mice (*Mus musculus*) has been linked to the nourishment levels of their mothers during gestation (Meikle, Kruper & Browning, 1995).

Maternal effects may be adaptive if mothers can use cues from current conditions to predict future environmental conditions to be encountered by their offspring and if offspring phenotype is altered accordingly (Bernardo, 1991). For example, female rotifers give birth to offspring with antipredator spines if the environment during gestation contains predators (Brody & Lawlor, 1984), while female aphids will give birth to winged daughters if maintained on poor food or housed under crowded conditions (Dixon, 1985). In situations where maternal and juvenile ecologies differ, maternal decisions about investment in offspring quality

may be determined during the juvenile phase of the mother, as has been demonstrated in cichlid fish (Taborsky, 2006).

A female can influence her offspring's phenotype and, therefore, its fitness at several different stages during reproduction: during development of the ovum, at mating/conception (attributes of the offspring's father if mate choice is operating), through pregnancy, when and where her clutch/litter is laid/born or through care after birth (Bernardo, 1996; Mousseau & Fox, 1998; Qvarnstrom & Price, 2001). In many species, paternal influence does not extend past genetic contributions upon fertilisation. Parental care by the father, whether it be male brooding of eggs as seen in pipefishes and seahorses (e.g. Wilson *et al.*, 2003), care of egg masses (e.g. Japanese goby, Fujii, Hironaka & Nomakuchi, 2005), or rearing of young as in many bird species (e.g. great tits, Isaksson, Uller & Andersson, 2006), is able to extend paternal influence on an offspring's phenotype and fitness beyond genetics. The carotenoid-based plumage coloration of nestling great tits (*Parus major*), which has implications for immune physiology and behaviour, is influenced by the colour of the rearing father, but not that of the rearing mother nor either of the genetic parents (Isaksson *et al.*, 2006).

Parental effects in viviparous lizards

One of the most significant influences on an offspring's phenotype that can be made by a mother is seen in viviparous species, in which young are retained *in utero* for the entire length of embryogenesis. Reptiles provide us with some special opportunities to investigate the maternal effects associated with viviparity. There have been over 100 separate origins of viviparity in squamates, more often than in all other vertebrate species combined (Blackburn, 1999). In conjunction with

reproductively bimodal species such as *Lacerta vivipara* (Arrayago, Bea & Heulin, 1996), *Lerista bougainvillii* (Qualls *et al.*, 1995) and *Saiphos equalis* (Smith & Shine, 1997), squamates provide an ideal group with which to study the evolution of viviparity.

There has been much research into the selective pressures leading to the evolution of viviparity. An early hypothesis, the “Cold-Climate” hypothesis (Weekes, 1935; Shine, 1985), was based on the observation that the proportion of viviparous reptilian taxa is higher in colder climates. This hypothesis suggested that in viviparous species maternal thermoregulation ensures that the developing embryo is exposed to higher temperatures than would be experienced by eggs in a nest, decreasing the time to hatching and enhancing the viability of the offspring (Shine, 1985). This hypothesis has recently been revised: it is now considered that diel distributions as well as mean incubation temperatures play a part in the selective forces for viviparity (Shine, 2004). This serves to explain the proliferation of viviparous taxa in warmer climates (Webb, Shine & Christian, 2006). The “Maternal Manipulation” hypothesis has therefore superseded the “Cold-Climate” hypothesis (Shine, 1995; Webb *et al.*, 2006). The view is that by manipulating thermal conditions during embryogenesis, mothers can enhance fitness-relevant phenotypic traits of their offspring (Shine, 1995). For example, pregnant death adders (*Acanthophis praelongus*) are able to maintain less variable body temperatures than nonpregnant females, presumably subjecting their offspring to less variable temperatures than would be experienced in a nest environment (Webb *et al.*, 2006). Females maintained at a diel range similar to the range selected by pregnant females produced offspring that were larger than females kept at a diel range similar to that selected by nonpregnant females. The fitness of their offspring was enhanced, as

larger offspring size was related to enhanced recapture probability in this study – presumably reflecting survival rates (Webb *et al.*, 2006).

In most squamates, viviparity is, in effect, prolonged egg retention, with simple placental arrangements (transfer of water, inorganic ions and probably a few organic nutrients) and the majority of embryonic nutrition is supplied through the yolk (Blackburn, 2000; Blackburn, Vitt & Beuchat, 1984). In a restricted number of squamates, placentotrophic nutrition becomes increasingly important, and is supported by a diverse range of placental arrangements (Blackburn, 2000; Blackburn *et al.*, 1984). Obviously placentotrophy (placental transfer of nutrients) must confer advantages that overcome the costs of producing and maintaining complex placentae: presumably these relate to ecological circumstances in which lecithotrophy (yolk provision of nutrients) has some limitations (Jones & Swain, 2006).

Two categories of placental nutritional provision, initially proposed by Stewart (1989), contribute to the diversity of modes of embryonic nutrition seen in squamates. Obligate placentotrophy is defined as placental provision that is required for the production of viable offspring. Facultative placentotrophy is defined as placental provision that supplements (enhances) embryonic nutrition, but which is not a requirement for successful production of viable offspring (Stewart, 1989; Thompson *et al.*, 1999b). These two forms of placentotrophy, which may involve provision of inorganic and/or organic nutrients, can function either to supplement, or to replace, yolk nourishment (Stewart, 1989), but are not mutually exclusive. Facultative placentotrophy has been documented in the predominantly lecithotrophic *Virginia stratula* (Stewart, 1989), *Niveoscincus metallicus* (Thompson *et al.*, 1999a) and the predominantly placentotrophic *Pseudemoia spenceri* (Thompson *et al.*, 1999c) and *P.pagenstecheri* (Thompson *et al.*, 1999b). Obligate placentotrophy has

been documented in several species, including *N. metallicus* (Thompson *et al.*, 1999a), *N. ocellatus* (Thompson *et al.*, 2001), *P. pagenstecheri* (Thompson *et al.*, 1999b), *P. entrecasteauxii* (Stewart & Thompson, 1993) and members of the genus *Mabuya* (Blackburn *et al.*, 1984).

Placentotrophy is thought to have first evolved as a facultative mechanism enabling mothers to supplement an adequate yolk supply (Stewart, 1989), thus enhancing offspring condition if circumstances are favourable during gestation (Swain & Jones, 2000a; Swain & Jones, 2000b; Thompson *et al.*, 1999a; Thompson *et al.*, 1999b; Thompson *et al.*, 1999c). It has previously been proposed that the major selective advantage arising from facultative placentotrophy is the introduction of flexibility into the timing of parturition (Jones & Swain, 2006). When, where and how mothers place their offspring is one of the most significant determinants of offspring success (Bernardo, 1996). In cold temperate and alpine habitats, climatic conditions are often subject to very rapid change, and periods of poor weather may last for days, or even weeks. Flexibility in birth date ensures that young are born into the most benign environment possible, enhancing post-natal survival rates and successful dispersal (Mathies & Andrews, 1995; Olsson & Shine, 1998; Rock, 2005; Swain & Jones, 2000a).

The capacity to ensure that young are born into the most benign environment possible is a previously unrecognised life history trait of great value to lizards living in cold climates (Olsson & Shine, 1998). Once the capacity for placental transfer was established, two evolutionary avenues became available: a) selection could exploit the capacity for deferred parturition in circumstances where this conferred major advantages (Chapter 2); or b) selection could lead to increasing dependence on placentotrophy with facultative transfer being replaced by an increasing obligate

component, and, it has been hypothesised, a reduced ability to defer parturition (Chapter 4) (Swain & Jones, ARC Discovery Grant 2002). Therefore, facultative placentotrophy must be seen as more than a transitional stage leading to obligate transfer, and more as a unique solution to the difficulties encountered by viviparous squamates living in unpredictable temperate climates (Swain & Jones, 2000a). I propose that the degree of facultative placentotrophy a species exhibits (Chapter 3: presence of facultative placentotrophy in *N. microlepidotus*) is related to its ability to defer parturition at the end of embryonic development. The major hypothesis to be explored in this thesis is that deferral of parturition after completion of embryonic development is a key strategy employed by females of viviparous lizards to maximise offspring fitness.

To gain an understanding of the evolution of placentotrophy and its hypothesised association with flexibility in the timing of parturition, the closely related species in the Australian genus *Niveoscincus* appear to be the best candidates available (Blackburn, 2000). The majority of viviparous lizards have a simple chorioallantoic placenta (described as Type I placenta, Stewart & Thompson, 1994). However, *Niveoscincus* sp. have placenta of intermediate complexity (Stewart & Thompson, 1994; Stewart & Thompson, 1998; Weekes, 1930), and species of the genus vary in both chorioallantoic placental complexity and the degree of placentotrophy (Thompson, Stewart & Speake, 2000; Thompson *et al.*, 2002). This genus has been well studied for the last two decades by researchers in Australia: there are extensive data on its reproductive ecology, endocrinology, placental structure and function, embryonic nutrition, and an established phylogeny (e.g. Jones & Swain, 1996; Melville & Swain, 2000; Olsson & Shine, 1999; Shine & Olsson, 2003; Swain & Jones, 1997). The majority of species within the genus exhibit annual reproduction

(Hutchinson, Swain & Driessen, 2001; Jones & Swain, 1996; Jones, Wapstra & Swain, 1997), in which females typically give birth in summer directly after completion of embryonic development; however, two species (*N. microlepidotus* and *N. greeni*) exhibit biennial reproduction, with females giving birth in the spring although embryonic development is completed in the autumn (Hutchinson, Robertson & Rawlinson, 1989; Olsson & Shine, 1998; Olsson & Shine, 1999). The well-studied species, *N. microlepidotus* (little published work is available on *N. greeni*), will be utilised to explore the consequences of deferral of parturition over winter (refer a above; Chapter 2). Decreased probability of survival of offspring born before winter has already been established (Olsson and Shine 1998); however, the effects on offspring characteristics of deferring parturition until spring have not been assessed to date. *Niveoscincus microlepidotus* appears to have a simple Type II placenta (J. Stewart pers. comm.) and ovulates a large yolky egg, suggesting that the species is primarily lecithotrophic, capable of facultative placental transfer but restricted obligate transfer (investigated in Chapter 3). Conversely the annually reproducing species *N. ocellatus* has a more complex Type II placenta, and has been shown to exhibit significant placentotrophy (Thompson *et al.*, 2000). This species will be utilised to investigate whether an increasing dependence on placentotrophy (obligate) results in a reduced ability to defer parturition (refer b above; Chapter 4). This experiment builds on earlier work on *N. metallicus* (Swain & Jones, 2000a): there are marked geographic differences in the timing of key events within the female reproductive cycle of *N. ocellatus* (Wapstra *et al.*, 1999), providing the opportunity for comparison between populations of the ability to defer parturition.

In addition to exploring the deferral of parturition over winter in *N. microlepidotus*, the effect of date of birth in spring on offspring characteristics will

be investigated in this biennial species (Chapter 3). Offspring phenotype and survival can be affected by oviposition (egg-laying) date, hatching date and birth date in reptiles (Blem & Blem, 1995; Civantos, Salvador & Veiga, 1999; Olsson & Shine, 1996; Olsson & Shine, 1997; Shine & Olsson, 2003; Sinervo & Doughty, 1996). In addition in viviparous species, experimental manipulation of maternal access to basking results in an increased range of dates over which neonates are born, and significant variation in offspring phenotype (Doughty & Shine, 1998; Mathies & Andrews, 1997; Shine & Downes, 1999; Shine & Harlow, 1993; Swain & Jones, 2000b; Wapstra, 2000).

How females are able to control the timing of parturition (Chapter 5) will be investigated in the second part of the thesis. Control of parturition is relatively well understood: the proximate endocrine control in reptiles is arginine vasotocin (Cree & Guillette, 1991), which stimulates the local effects of prostaglandins. For deferral of parturition to occur so that offspring are born into a more benign environment, there must be some environmental modulation of either the endocrine cascade initiating parturition or of oviductal innervation (Guillette, Dubois & Cree, 1991; Jones & Baxter, 1991). In the annually reproducing species *N. ocellatus*, the completion of embryonic development and the timing of parturition are closely associated. If parturition can be deferred beyond the completion of embryonic development, through what mechanism is this achieved? In biennially reproducing species, such as *N. microlepidotus*, the completion of embryonic development is separated from the timing of parturition by approximately 7-8 months (Girling, Jones & Swain, 2002). This provides a useful model for examining the potential role of the environment and/or β -adrenergic stimulation in the control of parturition, with the complicating

role of the embryonic-maternal interaction in controlling the timing of parturition (Challis *et al.*, 2001) removed.

Finally, paternal contributions to offspring fitness will be investigated in the third part of the thesis. The majority of squamate (lizards, snakes and amphisbaenians) reptile species exhibit no parental care; in one of the few examples in squamates in which parental care has been demonstrated, care relates to protection of the young (O'Connor & Shine, 2004). Parental effects can only, therefore, exert their influence until the time of oviposition or parturition, and the male's only contribution is a genetic one at the time of mating. In the context of this study, these nuclear genetic effects will be referred to as paternal effects (Bernardo, 1996). Determinants of male reproductive success include body size, territory/home range attributes, 'contest attributes', alternative mating tactics and female choice (Olsson & Madsen, 1998). Home ranges of mature male and female *N. ocellatus* and proximity data will be determined to identify available mates for each female. Genetic data will then be utilised to determine paternity and levels of multiple paternity within the population. This information, in conjunction with male characteristics measured, will identify determinants of male reproductive success in the species (Chapter 6a). Work on *Uta stansburiana* has demonstrated that the size of fathers within a litter can have an effect: offspring sired by the larger male were larger and in better condition than offspring sired by the smaller male; in addition, offspring sired by the larger male were more likely to be sons, but more daughters were sired by the smaller male (Calsbeek & Sinervo, 2002). In addition, genetic determinants of offspring dispersal are significant in both *U. stansburiana* (Sinervo *et al.*, 2006) and *Sceloporus occidentalis* (Massot *et al.*, 2003). Natal dispersal is important for both inbreeding avoidance and competition for resources (Clobert *et al.*, 2001), which can have

important consequences for offspring survival and fitness. Further information on the effect of paternal body size, within a litter, on offspring characteristics and parental and offspring characteristics on offspring survival will be explored in Chapter 6b. The section described here was omitted from the submitted manuscript (Chapter 6a) to provide that paper with a stronger focus for publishing purposes.

Outline of the thesis

Chapter 1: Introduction, and overview of the thesis.

Part 1. Deferral of parturition as a strategy for optimising offspring fitness in viviparous lizards.

Chapter 2: Explores the costs and benefits associated with the naturally protracted deferral of parturition by the southern snow skink *Niveoscincus microlepidotus*, and the female's ability to defer parturition in spring in response to adverse environmental conditions.

Chapter 3: Investigates the effect of the day of birth (for naturally born offspring in spring) on offspring characteristics in *N. microlepidotus*. This paper also reports upon the degree of placentotrophic contribution to embryonic nutrition in this species.

Chapter 4: Examines the ability of the spotted snow skink *Niveoscincus ocellatus* to delay parturition at the end of gestation, and the implications of such deferral for offspring quality and survival.

Part 2. Endocrine mechanisms controlling parturition and their modulation by environmental influences or beta-adrenergic stimulation.

Chapter 5: Investigates the endocrine mechanisms that control parturition in *N. microlepidotus* and *N. ocellatus*, and their modulation by a key environmental parameter (temperature) and the β -adrenergic system.

Part 3. Genetic control of offspring fitness – paternal influence.

Chapter 6a: Assesses female access to males and their subsequent reproductive success in *N. ocellatus*, and investigates genetic and spatial methods for determining paternity.

Chapter 6b: Explores paternal effects on offspring quality and survival in *N. ocellatus*.

Appendix 1: Treatment of a mite infestation in gravid spotted snow skinks (*N. ocellatus*).

This appendix resulted from an unsuccessful experiment that was not included in Chapter 4.

Appendix 2: A preliminary investigation into the effect of temperature on the induction of parturition by AVT in *N. ocellatus*.

This appendix reports on the results of a preliminary investigation that established the experimental parameters used in Experiment 2 in Chapter 5.

Presentation of the thesis

I have prepared the data chapters within this thesis as stand-alone scientific papers that have been submitted, or accepted, for publication. I am the primary author on all manuscripts, having undertaken the data collection, data analysis, and preparation of manuscripts; however, I have recognised the contributions of others by acknowledging them as co-authors. Publication status and authorship of individual manuscripts varies, and details are provided at the beginning of each chapter.

By necessity, there is repetition of some introductory, study species and bibliographic descriptions between data chapters. In addition, abstracts are included within each chapter, with the thesis abstract providing a broader summary of the main thesis findings. Formatting between chapters is necessarily not uniform because of the requirements of different journals. The content of each manuscript remains as submitted or as accepted for publication where relevant.

References

- Arrayago M-J, Bea A, Heulin B. 1996.** Hybridization experiments between oviparous and viviparous strains of *Lacerta vivipara*; a new insight into the evolution of viviparity in reptiles. *Herpetologica* **52**: 333-342.
- Bernardo J. 1991.** Manipulating egg size to study maternal effects on offspring traits. *Trends in Ecology and Evolution* **6**: 1-2.
- Bernardo J. 1996.** Maternal effects in animal ecology. *American Zoologist* **36**: 83-105.
- Blackburn DG. 1999.** Are viviparity and egg-guarding evolutionarily labile in squamates? *Herpetologica* **55**: 556-573.
- Blackburn DG. 2000.** Reptilian viviparity: past research, future directions, and appropriate models. *Comparative Biochemistry and Physiology Part A* **127**: 391-409.
- Blackburn DG, Vitt LJ, Beuchat CA. 1984.** Eutherian-like reproductive specialisations in a viviparous reptile. *Proceedings of the National Academy of Science, USA* **81**: 4860-4863.
- Blem CR, Blem LB. 1995.** The eastern cottonmouth (*Agkistrodon piscivorus*) at the northern edge of its range. *Journal of Herpetology* **29**: 391-398.
- Brody MS, Lawlor LR. 1984.** Adaptive variation in offspring size in the terrestrial isopod, *Armadillidium vulgare*. *Oecologia* **61**: 55-59.
- Calsbeek R, Sinervo B. 2002.** Uncoupling direct and indirect components of female choice in the wild. *Proceedings of the National Academy of Science, USA* **99**: 14897-14902.

-
- Challis JRG, Alfaidy N, Lye SJ, Gibb W, Patel FA, Gupta S, Martin R, Giannoulas D, Whittle WL, Xu P. 2001.** Endocrine and paracrine regulation of birth. *Biology of Reproduction* **64**: 104-104.
- Civantos E, Salvador A, Veiga JP. 1999.** Body size and microhabitat affect winter survival of hatchling *Psammodromus algirus* lizards. *Copeia* **1999**: 1112-1117.
- Clobert J, Danchin E, Dhondt AA, Nichols JD. 2001.** *Dispersal*. Oxford University Press, Oxford.
- Cree A, Guillette LJ Jr. 1991.** Effect of β -adrenergic stimulation on uterine contraction in response to arginine vasotocin and prostaglandin $F_{2\alpha}$ in the gecko *Hoplodactylus maculatus*. *Biology of Reproduction* **44**: 499-510.
- Dixon AFG. 1985.** *Aphid ecology*. Blackie, Glasgow.
- Doughty P, Shine R. 1998.** Reproductive energy allocation and long-term energy stores in a viviparous lizard (*Eulamprus tympanum*). *Ecology* **79**: 1073-1083.
- Fujii S, Hironaka M, Nomakuchi S. 2005.** Male body size and brooding success under natural conditions in the freshwater Japanese goby, *Rhinogobius* sp OR (orange type). *Journal of Ethology* **23**: 127-132.
- Girling JE, Jones SM, Swain R. 2002.** Delayed ovulation and parturition in a viviparous alpine lizard (*Niveoscincus microlepidotus*): morphological data and plasma steroid concentrations. *Reproduction Fertility and Development* **14**: 43-53.
- Guillette LJ Jr, Dubois DH, Cree A. 1991.** Prostaglandins, oviductal function, and parturient behaviour in nonmammalian vertebrates. *American Journal of Physiology* **260**: R854-R861.

-
- Hutchinson MN, Robertson P, Rawlinson PA. 1989.** Redescription and ecology of the two endemic Tasmanian scincid lizards *Leiopisma microlepidotum* and *L. pretiosum*. *Papers and Proceedings of the Royal Society of Tasmania* **123**: 257-274.
- Hutchinson MN, Swain R, Driessen M. 2001.** *Snakes and lizards of Tasmania*. Nature conservation branch, Department of Primary Industries, Water and Environment and University of Tasmania, Hobart.
- Isaksson C, Uller T, Andersson S. 2006.** Parental effects on carotenoid-based plumage coloration in nestling great tits, *Parus major*. *Behavioral Ecology and Sociobiology* **60**: 556-562.
- Jones RE, Baxter DC. 1991.** Gestation, with emphasis on corpus luteum biology, placentation, and parturition. In: Pang PKJ and Schreibnz MP, eds. *Vertebrate endocrinology: fundamentals and biomedical implications*.
- Jones SM, Swain R. 1996.** Annual reproductive cycle and annual cycles of reproductive hormones in plasma of female *Niveoscincus metallicus* from Tasmania. *Journal of Herpetology* **30**: 140-146.
- Jones SM, Swain R. 2006.** Placental transfer of H-3-oleic acid in three species of viviparous lizards: a route for supplementation of embryonic fat bodies? *Herpetological Monographs*: 186-193.
- Jones SM, Wapstra E, Swain R. 1997.** Asynchronous male and female gonadal cycles and plasma steroid concentrations in a viviparous lizard, *Niveoscincus ocellatus* (Scincidae), from Tasmania. *General and Comparative Endocrinology* **108**: 271-281.

Lindholm AK, Hunt J, Brooks R. 2006. Where do all the maternal effects go?

Variation in offspring body size through ontogeny in the live-bearing fish

Poecilia parae. *Biology Letters* **2**: 586-589.

Massot M, Clobert J. 2000. Processes at the origin of similarities in dispersal

behaviour among siblings. *Journal of Evolutionary Biology* **13**: 707-719.

Massot M, Huey RB, Tsuji JS, van Berkum FH. 2003. Genetic, prenatal, and

postnatal correlates of dispersal in hatchling fence lizards (*Sceloporus*

occidentalis). *Behavioural Ecology* **14**: 650-655.

Mathies T, Andrews RM. 1995. Thermal and reproductive biology of high and low

elevation populations of the lizard *Sceloporus scalaris* - implications for the

evolution of viviparity. *Oecologia* **104**: 101-111.

Mathies T, Andrews RM. 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.

Meikle DB, Kruper JH, Browning CR. 1995. Adult male house mice born to

undernourished mothers are unattractive to estrous females. *Animal*

Behaviour **50**: 753-758.

Melville J, Swain R. 2000. Mitochondrial DNA-sequence based phylogeny and

biogeography of the snow skinks (Squamata: Scincidae: *Niveoscincus*) of

Tasmania. *Herpetologica* **56**: 196-208.

Mousseau TA, Fox CW. 1998. The adaptive significance of maternal effects.

Trends in Ecology & Evolution **13**: 403-407.

O'Connor DE, Shine R. 2004. Parental care protects against infanticide in the lizard

Egernia saxatilis (Scincidae). *Animal Behaviour* **68**: 1361-1369.

-
- Olsson M, Madsen T. 1998.** Sexual selection and sperm competition in reptiles. In: Birkhead TR and Moller AP, eds. *Sperm competition and sexual selection*. Cambridge: Academic press. 503-578.
- Olsson M, Shine R. 1996.** Does reproductive success increase with age or with size in species with indeterminate growth? A case study using sand lizards (*Lacerta agilis*). *Oecologia* **105**: 175-178.
- Olsson M, Shine R. 1997.** The seasonal timing of oviposition in sand lizards (*Lacerta agilis*): why early clutches are better. *Journal of Experimental Zoology* **10**: 369-381.
- Olsson M, Shine R. 1998.** Timing of parturition as a maternal care tactic in an alpine lizard species. *Evolution* **52**: 1861-1864.
- Olsson M, Shine R. 1999.** Plasticity of frequency of reproduction in an alpine lizard, *Niveoscincus microlepidotus*. *Copeia* **1999**: 794-796.
- Qualls CP, Shine R, Donellan S, Hurchinson M. 1995.** The evolution of viviparity within the Australian scincid lizard *Lerista bougainvilli*. *Journal of Zoology (London)* **237**: 13-26.
- Qvarnstrom A, Price TD. 2001.** Maternal effects, paternal effects and sexual selection. *Trends in Ecology & Evolution* **16**: 95-100.
- Reid JM, Arcese P, Keller LF, Hasselquist D. 2006.** Long-term maternal effect on offspring immune response in song sparrows *Melospiza melodia*. *Biology Letters* **2**: 573-576.
- Reinhold K. 2002.** Maternal effects and the evolution of behavioral and morphological characters: a literature review indicates the importance of extended maternal care. *Journal of Heredity* **93**: 400-405.

-
- Rock J. 2005.** Delayed parturition: constraint or coping mechanism in a viviparous gekkonid? *Journal of Zoology (London)* **268**: 355-360.
- Shine R. 1985.** The evolution of viviparity in reptiles: an ecological analysis. In: Gans C and Billett F, eds. *Biology of the Reptilia*. New York: John Wiley and sons. 605-695.
- Shine R. 1995.** A new hypothesis for the evolution of viviparity in reptiles. *The American Naturalist* **145**: 809-823.
- Shine R. 2004.** Does viviparity evolve in cold climate reptiles because pregnant females maintain stable (not high) body temperatures? *Evolution* **58**: 1809-1818.
- Shine R, Downes SJ. 1999.** Can pregnant lizards adjust their offspring phenotypes to environmental conditions? *Oecologia* **119**: 1-8.
- Shine R, Harlow P. 1993.** Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia* **96**: 122-127.
- Shine R, Olsson M. 2003.** When to be born? Prolonged pregnancy or incubation enhances locomotor performance in neonatal lizards (Scincidae). *Journal of Evolutionary Biology* **16**: 823-832.
- Sinervo B, Calsbeek R, Comendant T, Both C, Adamopoulou C, Clobert J. 2006.** Genetic and maternal determinants of effective dispersal: the effect of sire genotype and size at birth in side-blotched lizards. *American Naturalist* **168**: 88-99.
- Sinervo B, Doughty P. 1996.** Interactive effects of offspring size and timing of reproduction on offspring reproduction: experimental, maternal, and quantitative genetic aspects. *Evolution* **50**: 1314-1327.

-
- Smith SA, Shine R. 1997.** Intraspecific variation in reproductive mode within the scincid lizard *Saiphos equalis*. *Australian Journal of Zoology* **45**: 435-445.
- Stewart JR. 1989.** Facultative placentotrophy and the evolution of squamate placentation: quality of eggs and neonates in *Virginia striatula*. *The American Naturalist* **133**: 111-137.
- Stewart JR, Thompson MB. 1993.** A novel pattern of embryonic nutrition in a viviparous reptile. *Journal of Experimental Biology* **174**: 97-108.
- Stewart JR, Thompson MB. 1994.** Placental structure of the Australian lizard, *Niveoscincus metallicus* (Squamata: Scincidae). *Journal of Morphology* **220**: 223-236.
- Stewart JR, Thompson MB. 1998.** Placental ontogeny of the Australian scincid lizards *Niveoscincus coventryi* and *Pseudemoia spenceri*. *Journal of Experimental Zoology* **282**: 535-559.
- Swain R, Jones SM. 1997.** Maternal transfer of 3H-labelled leucine in the viviparous lizard *Niveoscincus metallicus* (Scincidae: Lygosominae). *Journal of Experimental Zoology* **277**: 139-145.
- Swain R, Jones SM. 2000a.** Facultative placentotrophy: half-way house or strategic solution? *Comparative Biochemistry and Physiology Part A* **127**: 441-451.
- Swain R, Jones SM. 2000b.** Maternal effects associated with gestation conditions in a viviparous lizard, *Niveoscincus metallicus*. *Herpetological Monographs* **14**: 432-440.
- Taborsky B. 2006.** Mothers determine offspring size in response to own juvenile growth conditions. *Biology Letters* **2**: 225-228.
- Thompson MB, Speake BK, Stewart JR, Russell K, McCartney RJ, Surai PF. 1999a.** Placental nutrition in the viviparous lizard *Niveoscincus metallicus*:

the influence of placental type. *Journal of Experimental Biology* **202**: 2985-2997.

Thompson MB, Speake BK, Stewart JR, Russell KJ, McCartney RJ. 2001.

Placental nutrition in the Tasmanian skink, *Nivescincus ocellatus*. *Journal of Comparative Physiology B* **171**: 155-160.

Thompson MB, Stewart JR, Speake BK. 2000. Comparison of nutrient transport

across the placenta of lizards differing in placental complexity. *Comparative Biochemistry and Physiology Part A* **127**: 469-479.

Thompson MB, Stewart JR, Speake BK, Hosie MJ, Murphy CR. 2002.

Evolution of viviparity: what can Australian lizards tell us? *Comparative Biochemistry and Physiology Part B* **131**: 631-643.

Thompson MB, Stewart JR, Speake BK, Russell K, McCartney RJ, Surai PF.

1999b. Placental nutrition in a viviparous lizard (*Pseudemoia pagenstecheri*) with a complex placenta. *Journal of Zoology, (London)* **248**: 295-305.

Thompson MB, Stewart JR, Speake BK, Russell KJ, McCartney RJ. 1999c.

Placental transfer of nutrients during gestation in the viviparous lizard, *Pseudemoia spenceri*. *Journal of Comparative Physiology B* **169**: 319-328.

Wapstra E. 2000. Maternal basking opportunity affects juvenile phenotype in a

viviparous lizard. *Functional Ecology* **14**: 345-353.

Wapstra E, Swain R, Jones SM, O'Reilly J. 1999. Geographic and annual variation

in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Australian Journal of Zoology* **47**: 539-550.

Webb JK, Shine R, Christian KA. 2006. The adaptive significance of reptilian

viviparity in the tropics: testing the maternal manipulation hypothesis. *Evolution* **60**: 115-122.

Weekes HC. 1930. On placentation in reptiles. *Proceedings of the Linnean Society of New South Wales* **55**: 550-576.

Weekes HC. 1935. A review of placentation among reptiles with particular regard to the function and evolution of the placenta. *Proceedings of the Zoological Society of London* **3**: 625-645.

Wilson AB, Ahnesjo I, Vincent ACJ, Meyer A. 2003. The dynamics of male brooding, mating patterns, and sex roles in pipefishes and seahorses (family Syngnathidae). *Evolution* **57**: 1374-1386.

Part 1.

*Deferral of parturition as a strategy for
optimising offspring fitness in viviparous
lizards.*

Chapter 2

Are babies better in autumn or spring? The consequences of extending gestation in a biennially reproducing viviparous lizard

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ABSTRACT

Niveoscincus microlepidotus, the southern snow skink, is a biennially reproducing alpine viviparous lizard with an extremely protracted gestation period: embryos are fully developed in autumn, but held over winter so that offspring are born in spring. The obvious benefits for offspring survival of delaying birth until spring have been demonstrated previously. To examine the consequences of deferred parturition for offspring characteristics, we compared neonates obtained in autumn by dissection with neonates born naturally in the spring.

Our results demonstrate that deferral of parturition until spring represents a trade-off between key offspring characteristics (spring neonates exhibit lower growth rates, reduced sprint speed after birth, reduced condition and decreased energy reserves) and offspring size (spring neonates are heavier (wet mass) and longer (snout-vent length)). Furthermore, when females are placed into cold experimental conditions in spring around the time of natural parturition, this species is able to

defer parturition for an additional 4 weeks with no significant effect on offspring characteristics. Our results provide further evidence that flexibility in birth date provides a significant advantage to viviparous lizards living in cold climates.

INTRODUCTION

Flexibility in birth date may enhance post-natal survival and dispersal of offspring, and, thus, enhance maternal fitness. Environmental factors influence the timing of parturition of many mammalian species including Dall's sheep, *Ovis dalli dalli*, in Alaska (Rachlow and Bowyer, '94); red squirrels in Canada (Reale et al., '03) and several bat species (Cumming and Bernard, '97; Arlettaz et al., '01). Furthermore, Dall's sheep may exhibit plasticity in patterns of maternal investment during gestation to compensate for variation in the timing of birth (Rachlow and Bowyer, '94).

In reptiles, there is considerable evidence that oviposition (egg-laying) date or birth date influence the probability of offspring survival (Blem and Blem, '95; Olsson and Shine, '96; Sinervo and Doughty, '96; Olsson and Shine, '97; Civantos et al., '99; Shine and Olsson, '03). In the genus *Niveoscincus*, a group of viviparous (live-bearing) skinks from cool temperate Tasmania, there are inter-specific differences in the ability to defer parturition if environmental conditions are poor at the time of completion of embryonic development (Swain and Jones, '00a; Atkins et al., in press - Ch. 4). In cold temperate and alpine habitats particularly, climatic conditions are often subject to very rapid change, and periods of poor weather may last for days, or even weeks. The capacity to ensure that young are born into the most benign environment possible is therefore a life history trait of great value to reptiles living in

cold and variable climates (Olsson and Shine, '98). The “maternal manipulation” hypothesis explains the flexibility in birth date which can be expressed by viviparous species (Shine, '95; Webb et al., '06); females may enhance the fitness of their offspring by manipulating thermal conditions during embryogenesis.

Niveoscincus microlepidotus (O'Shaughnessy, 1874), the southern snow skink, is an alpine viviparous lizard with biennial reproduction that represents an extreme ability to defer parturition (Hutchinson et al., '89; Olsson and Shine, '98; '99). Ovulation occurs in spring, and embryonic development continues over summer. By late autumn, the embryos are fully formed, have used nearly all their yolk and have sizeable abdominal fat bodies (Girling et al., '02a; '02b) but parturition is delayed until late in the following spring, so that gestation takes approximately a year to complete.

This form of biennial reproductive cycle, with such a protracted gestation (as opposed to an extended vitellogenesis), is rare among viviparous lizards: the alpine *N. greeni* from Tasmania (Hutchinson et al., '89), a subalpine population of the New Zealand common gecko, *Hoplodactylus maculatus*, from Macraes Flat, Central Otago (Cree and Guillette, '95), and the Central American lizard *Barisia monticola* exhibit similar reproductive cycles (Vial and Stewart, '85). In such species the evolution of a biennial reproductive cycle has been attributed to cool/cold climates and short activity seasons (Vial and Stewart, '85; Hutchinson et al., '89; Cree and Guillette, '95; Olsson and Shine, '98): females effectively manipulate birth date so that young are born at a more suitable time of the year. However, in *N. microlepidotus* at least, the reproductive cycle is plastic, and governed by proximate environmental conditions: preovulatory females can be ‘forced’ through gestation in approximately 4 months by maintaining females in laboratory conditions with

continuous access to heat (Olsson and Shine, '98; '99). Furthermore, the embryos of females captured from the wild in autumn survive for at least several days in the laboratory if delivered by dissection, or if parturition is induced by arginine vasotocin (AVT) (Girling et al., '02a; '02b), although neonates produced in autumn by an artificially shortened gestation do not survive the winter if released into the wild shortly after birth (Olsson and Shine, '98).

We hypothesise that the biennial reproductive cycle of *N. microlepidotus* reflects a trade-off between the obvious advantage (Olsson and Shine, '98) of ensuring young are born into a benign (spring) environment and the costs, to the embryos, of the protracted gestation. Our first aim is, therefore, to compare phenotypic characteristics, performance and growth rates of neonates produced by dissection in the laboratory in autumn with those of neonates born naturally in spring. This will allow us to assess the impact of the prolonged retention upon the fully developed embryos.

However, even after emergence in spring, females of *N. microlepidotus* may further trade off neonatal quality against probability of survival after birth: they do not give birth until several weeks after spring emergence (Olsson and Shine, '99), and there is a wide (up to 6 weeks) spread of parturition dates (Olsson and Shine, '98; Shine and Olsson, '03). We further investigate our hypothesis by examining the effects of experimental manipulation of the maternal environment during the final stages of gestation on offspring phenotype and performance variables. Will additional costs be incurred in terms of offspring quality if females are forced to defer parturition until later in spring in response to unfavourable environmental conditions?

MATERIALS AND METHODS

Female *Niveoscincus microlepidotus* were collected by hand, noosing or mealworm ‘fishing’ from near the summit (1270 m) of Mt. Wellington, Hobart, Tasmania, Australia (42°53’S, 147°14’E). As this species exhibits a biennial reproductive cycle, females in one of two reproductive conditions are present in the population at any one time, so reproductive status was determined by gentle palpation (Girling et al., '02a). All animals brought into the laboratory were treated for mites by dipping in Neguvon[®], with regular treatments at 2-weekly intervals (Atkins and Wapstra, '04 - App. 1). Lizards were maintained in pairs in plastic containers (30 cm x 20 cm x 10 cm) in an air-conditioned room provided with an 11:13 h light:dark regime at natural light intensities and a background temperature of 12 °C (standard laboratory conditions). Each container had a basking area, retreat site, and a layer of paper pellets. A thermal gradient (12 – 35 °C) was obtained through a low wattage light bulb (on 09:00 – 17:00) positioned over one end of the cage. Containers were repositioned within the room several times weekly to minimise position effects. Lizards were fed with *Tenebrio* larvae (mealworms), meaty cat food or pear-banana baby food three times per week. A calcium supplement was dusted over the food and multi-vitamin supplements were supplied in the drinking water, which was available *ad libitum*. Maternal size (snout-vent length - SVL) and mass were recorded post-partum.

CONSEQUENCES OF EXTENDING GESTATION: COMPARISON OF NEONATES BORN IN AUTUMN OR SPRING OF THE SAME YEAR

- ‘BIRTHS’ IN AUTUMN

Pregnant females were collected before hibernation (approximately 6-7 months prior to expected time of parturition) in March (austral autumn) 2003 (N = 21) and 2004 (N = 20). They were held without access to basking lights as some females may give birth at this time of year if brought into laboratory conditions (N. Atkins and S.M. Jones personal observations). Females were chilled and killed by decapitation (within several days of capture), and the fully developed embryos were removed from the oviducts by dissection. Offspring were maintained and their key characteristics measured as described below.

- NATURAL BIRTHS IN SPRING

Pregnant females near parturition (N = 40) were collected in October (austral spring) of 2003 and brought into the laboratory. The females (N = 20) used in 2004 were from the control group of the deferral experiment (described below). Containers were checked for births several times per day. Offspring were maintained and their key characteristics measured as described below.

- ‘BIRTHS’ IN SPRING

To investigate the effect of delivery by dissection on offspring characteristics, pregnant females near parturition (N = 21) were collected in October of 2004. After the first female had given birth naturally, embryos of all remaining females were delivered by dissection of females killed by decapitation. The neonates were compared with the naturally born neonates of that year. Offspring were maintained and their key characteristics measured as described below.

ABILITY TO DEFER PARTURITION IN SPRING: EXPERIMENTAL MANIPULATION OF MATERNAL ENVIRONMENT

Pregnant females near parturition ($N = 40$) were collected in October 2004, and maintained in pairs in the laboratory. After the first birth in captivity (suggesting that the remainder of the females were close to giving birth), half the females (housed in pairs) were moved to a room maintained at 5 °C (mean maximum temperature was 5.3 °C on Mt Wellington in October 2003: Australian Bureau of Meteorology), with room lighting (10:14, light: dark), but no basking light, for 4 weeks. These lizards were supplied with water *ad libitum* but no food. The other females (controls) remained in the standard laboratory conditions (as described above), housed in pairs.

All females were checked several times daily for births. After 4 weeks, females held at 5 °C were returned to the standard laboratory conditions. The day that the first female gave birth was designated day 1, and for subsequent births, the date of birth was recorded as days since day 1. Offspring were maintained and their key characteristics measured as described below.

OFFSPRING MAINTENANCE AND MEASUREMENT OF KEY CHARACTERISTICS: ALL EXPERIMENTS

For litters of two or more neonates (litter size \pm SE: 2.5 ± 0.15 , range: 1-4), one neonate was killed on the day of birth, its mass and SVL were recorded and the abdominal fat bodies removed by dissection. The carcass of the neonate and its fat bodies were dried (dried in an oven at ~ 70 °C for 5 days) and their dry weights determined.

Birth mass, SVL, tail length, and sprint speed were measured for remaining neonates. A measure of body condition was obtained by generating the Student residuals from a mass - SVL regression (generated using data from all neonates). Sprint speed was measured using a temperature controlled (26 °C) computerised racetrack with three sets of photoelectric detectors. Animals were first warmed to 26 °C for 30 min (Melville, '98) and were induced to run down the 1 m track by a soft paintbrush. Each lizard was run twice, with no rest between runs. Two times were obtained from each run, with the fastest speed (cm/s) of the four results being used for analysis.

For experiments conducted in 2003, all neonates were further assessed for post-natal growth, and basking frequency. Neonates were individually marked with non-toxic silver paint, and were allocated randomly to groups of five. They were kept under standard laboratory conditions for 11 weeks after birth. To minimise position effects within the laboratory, containers were repositioned each time the lizards were fed. Neonates were fed three times per week with a variety of meaty cat food or pear-banana baby food, and nematodes (white worms). All neonates were periodically given a calcium supplement in their food; multi-vitamin supplements were available in the drinking water, which was available *ad libitum*. Dominance hierarchies were not established within these groups, and animals shared the basking site with no apparent conflict. Mass, lengths (SVL and tail) and sprint speed were measured every second week for 11 weeks (neonates that did not survive this entire period were excluded from analyses). During this period the size variables increased linearly: consequently, the slopes of the linear regressions of mass (or either of the length measurements) against time were taken as indices of growth (Swain and Jones, '00b). Sprint speed data showed no trend with time and therefore all data were

combined to obtain an average response for each litter. Basking frequency was assessed in alternate weeks by rapid surveying of cages at 15 min intervals between 10 am and 4 pm (one day every 2 weeks), and noting which animals were basking (25 observations for each day). Basking frequency of individuals, also, did not change between birth and the end of the experiment; so all observations were combined to obtain an average basking response for each litter. At the end of the 11 weeks, neonates were released either immediately (spring births) or after winter (autumn births) at the site of maternal capture.

STATISTICAL ANALYSIS

All statistical analyses were carried out in SAS (v 6.12). To avoid the problems of non-independence of data derived from siblings, all analyses were carried out on litter means. Where significant differences were detected using ANOVA, differences between groups (pre-planned comparisons) were analysed using the CONTRAST statement, and where significant differences were detected in an ANCOVA, only pre-planned comparisons were used. Homogeneity of group variances was assessed by studying a plot of standard deviations against means, while examining plots of standardized residuals against estimated values and the normal probability curve of the residuals checked assumptions of normality. When necessary, data were log-transformed to stabilize variances and/or normalize data.

Neonates that were found at birth with part of their tail missing were excluded from analyses including birth weight or tail length. When tail loss occurred after the day of birth, data for mass growth and tail growth were excluded from the analyses but not data for SVL: caudal autotomy at birth does not inhibit growth measured as an increase in SVL (Chapple et al., '04).

RESULTS

EFFECT OF EXTENDING GESTATION ON OFFSPRING CHARACTERISTICS

For experiments conducted in 2003, autumn neonates were compared with neonates naturally born in the following spring (Figs. 1 and 2). In 2004, autumn neonates were compared with neonates born naturally (control group of deferral experiment) in the spring (Fig. 3).

- COMPARISON OF AUTUMN NEONATES AND NATURALLY BORN NEONATES

Overall, deferring parturition until spring has mixed consequences for the offspring. In both years of our study, naturally born neonates were significantly heavier (wet mass) than autumn neonates (ANCOVA (maternal mass as covariate), $F_{(5,139)} = 3.9$, $P = 0.002$, log transformed), in 2003 ($P = 0.003$) and 2004 ($P = 0.003$). However, there was no effect of over-wintering on the dry mass of neonates or sprint speed at birth ($P > 0.05$). Naturally born neonates were significantly longer (SVL) than autumn neonates in both years (ANOVA, $F_{(5,131)} = 15.52$, $P = 0.0001$), in 2003 ($P = 0.0001$) and in 2004 ($P = 0.0001$), but there was no significant difference in tail length between the two sets of neonates in either year ($P > 0.05$). The condition of naturally born neonates was significantly lower than that of autumn neonates in 2003 (ANOVA, $F_{(5,158)} = 6.37$, $P = 0.0001$) (group comparison $P = 0.0001$) but not in 2004 ($P > 0.05$). Energy stores (i.e. dry mass of abdominal fat bodies) were significantly lower in naturally born neonates than autumn neonates (ANCOVA (neonate mass as covariate), $F_{(5,127)} = 16.00$, $P = 0.0001$) in 2003 ($P = 0.0001$) and in 2004 ($P = 0.03$).

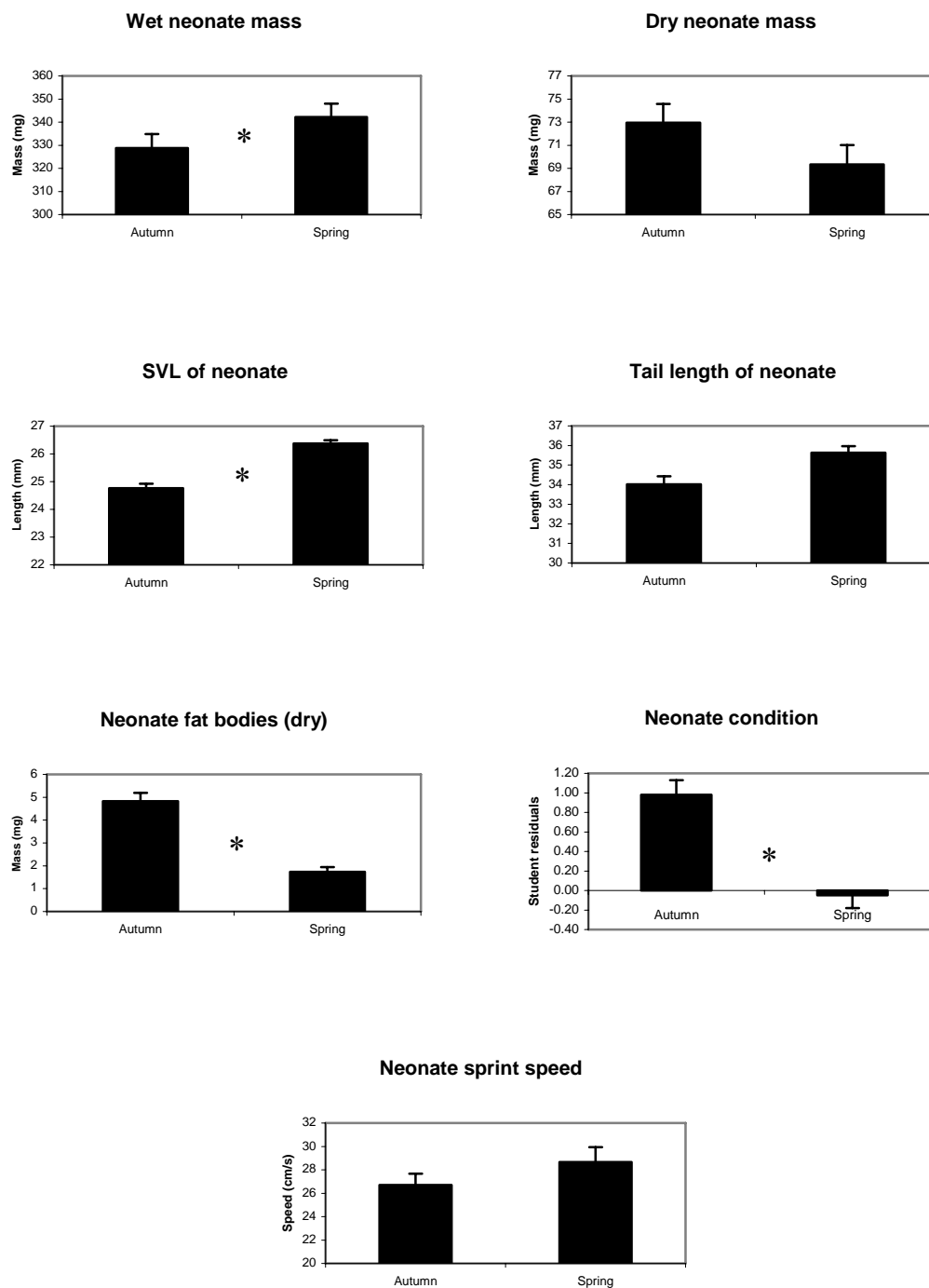


Fig. 1. Comparison of offspring characteristics and performance variables of autumn (delivered by dissection) and spring born *Niveoscincus microlepidotus* neonates, in 2003 at birth (litter means). Error bars are one standard error of the mean. * represents a significant statistical difference ($P < 0.05$).

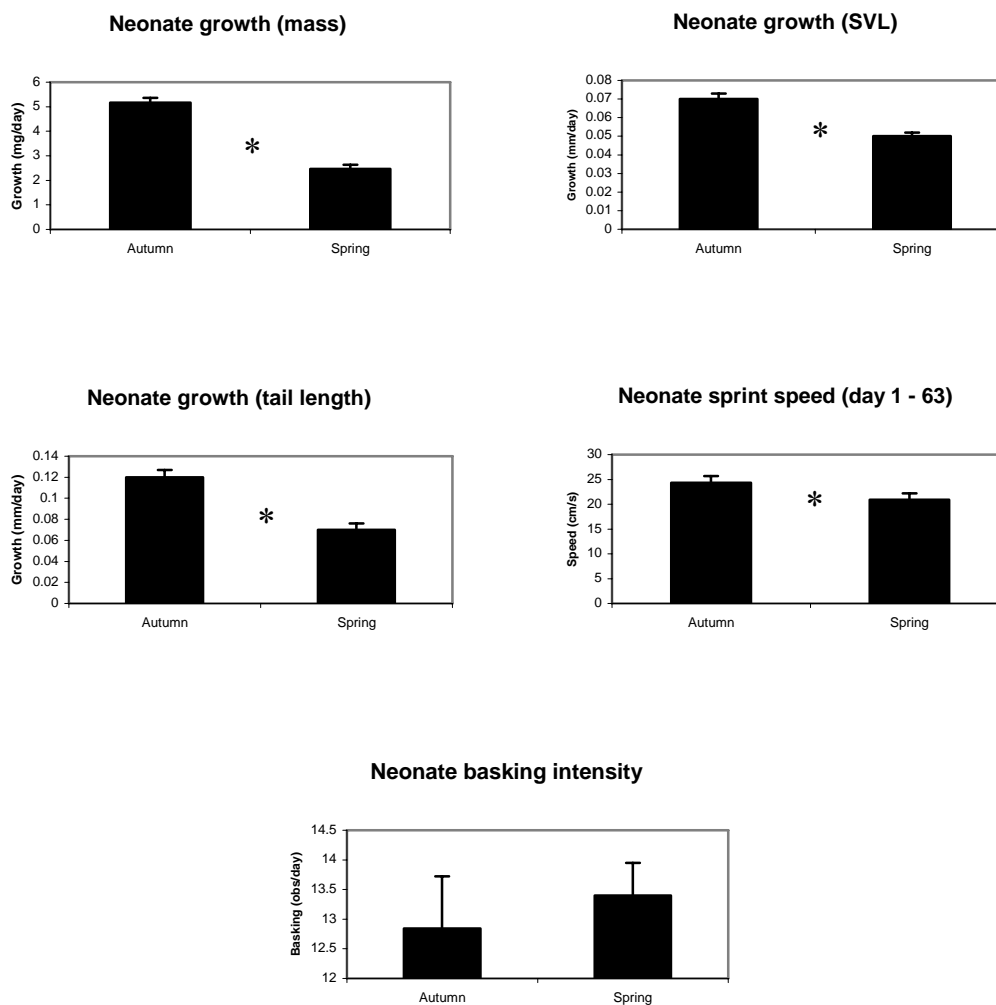


Fig. 2. Comparison of growth and performance variables of autumn (delivered by dissection) and spring born *Niveoscincus microlepidotus* neonates in 2003 (litter means). Error bars are one standard error of the mean. * represents a significant statistical difference ($P < 0.05$).

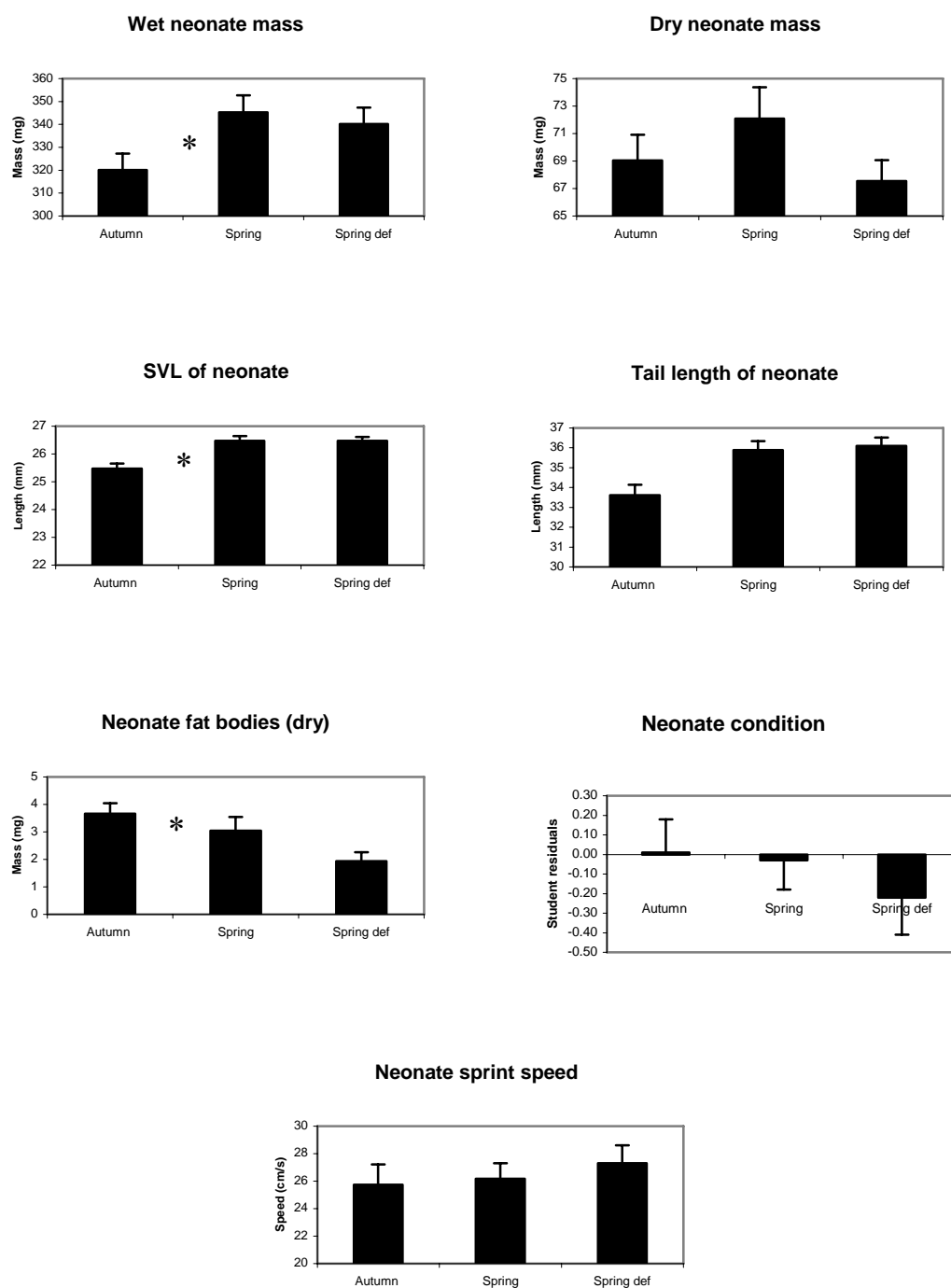


Fig. 3. Comparison of offspring characteristics and performance variables of autumn (delivered by dissection) and spring born *Niveoscincus microlepidotus* neonates, in 2004 at birth (litter means). Error bars are one standard error of the mean. * represents a significant statistical difference ($P < 0.05$).

Differences in neonate growth and performance criteria were assessed in 2003 (Fig. 2). Rate of increase of mass (ANOVA, $F_{(1,40)} = 114.19$, $P = 0.0001$), of SVL (ANOVA, $F_{(1,56)} = 34.54$, $P = 0.0001$) and tail length (ANCOVA (initial tail length as covariate) $F_{(2,43)} = 32.41$, $P = 0.0001$) was significantly lower for naturally born neonates than for autumn neonates. After 2 months, autumn neonates were significantly heavier than naturally born neonates (autumn: 626.6 ± 15.57 mg (19), spring: 498.6 ± 15.04 mg (35); ANOVA, $F_{(1,52)} = 26.32$, $P = 0.0001$), but there was no significant difference in SVL (autumn: 29.86 ± 0.22 mm (19), spring: 29.57 ± 0.21 mm (40); $P > 0.05$). Sprint speed, measured in the 2 months after birth, was slower for naturally born neonates than for neonates ‘born’ in autumn (ANOVA, $F_{(1,57)} = 5.12$, $P = 0.03$, log transformed) in 2003. Basking intensity did not differ significantly between autumn and naturally born neonates ($P > 0.05$).

- EFFECT OF DELIVERY BY DISSECTION ON NEONATE CHARACTERISTICS AT BIRTH

Delivery by dissection had minimal effects on the neonates. Neonates delivered by dissection in the spring of 2004 were significantly shorter (SVL, 25.88 ± 0.23 mm (19)) than neonates born naturally in that season (26.47 ± 0.17 mm (20); ANOVA, $F_{(1,37)} = 4.47$, $P = 0.041$), however, there were no other significant differences in any variable measured ($P > 0.05$) between neonates delivered by dissection in spring and naturally born neonates from the same season.

EFFECT OF DEFERRING PARTURITION IN SPRING

When pregnant females were placed into cold conditions at the expected time of parturition, the date of parturition was significantly delayed but there were no

measurable effects on the offspring. The first birth in the experimental group was delayed by 30 days (approximately the length of time spent in cold conditions) relative to the first birth among the control females: all control females gave birth at least two weeks before any of the deferred group. There was no significant effect of treatment ($P > 0.05$) (4 weeks at 5 °C) on any offspring characteristic or performance measure (Fig. 3). All neonates produced by females in both the experimental group and the control group were viable and survived until they were released two weeks after birth.

DISCUSSION

In cool-temperate climates, reptilian neonates experience an obvious advantage if they are born at an equable time of year. In some viviparous species, females are able to manipulate parturition date to ensure this occurs. However, a closer examination of the proximate consequences of the timing of parturition for offspring characteristics reveals a more complex story. For *Niveoscincus microlepidotus*, the protracted gestation extending over winter and into spring means that spring neonates have reduced fat body stores, decreased condition, decreased growth potential and reduced sprint speed after birth compared with neonates delivered in autumn by dissection. These results clearly support our hypothesis: the survival advantage of being born into benign spring conditions does come at some cost to the neonates.

Despite this proximate effect on the neonates, spring-born offspring that survive may expect several months of equable weather to grow and accumulate further energy reserves before entering hibernation the following winter. In contrast, offspring of congeneric annually reproducing species, with reduced abilities to defer

parturition in response to cold conditions (Swain and Jones, '00a; Atkins et al., '06 - Ch. 3), are born in summer, and may have only two months available for growth before their first hibernation (Jones and Swain, '96; Wapstra et al., '99). Furthermore, in *N. microlepidotus*, delaying birth until spring results in larger neonates: spring neonates are heavier (wet weight) and longer than neonates produced in autumn. In reptiles, larger neonatal size is often an indicator of quality (Bernado, '96) and a strong indicator of the probability of survival (e.g. Fox, '78; Ferguson and Fox, '84; Sinervo and Doughty, '96; Civantos et al., '99) and has been suggested to be a benefit of delaying the birth of fully developed reptilian embryos (Shine and Olsson, '03). However, in the gecko *H. maculatus*, Rock ('05) found no increase in body mass or size, nor any difference in sprint speeds, between neonates produced by AVT-induced parturition in either autumn or spring, after females experienced a natural gestation in the wild. Although our results clearly indicate additional embryonic growth over winter, in contrast, Olsson and Shine ('98) found no difference in mass and SVL between autumn and spring neonates of *N. microlepidotus*, while Girling et al. ('02a) found no significant change in mass of embryos over winter. These conflicting results may reflect subtle differences between these studies in the thermal environments experienced by the gestating mothers. Olsson and Shine ('98; '99) forced females through a rapid gestation to produce neonates in autumn, while Girling et al. ('02a) removed embryos from females collected in summer and kept in artificial hibernation over winter. In contrast, our results were obtained from females that were collected from the wild shortly before the neonates were obtained, and, furthermore, we demonstrated that obtaining neonates by dissection rather than natural birth had minimal effect on the offspring.

The protracted gestation in *N. microlepidotus* imposes significant energetic costs on the embryos and, presumably, the gestating females. How are these embryos nourished/supported during this long gestation? Our results clearly demonstrate that embryonic fat stores are depleted over winter. Placental supplementation of embryonic fat reserves is possible: we have demonstrated placental transfer of lipid (^3H -oleic acid) into embryos and embryonic abdominal fat bodies in females of *N. microlepidotus* collected in late summer and in autumn (Jones and Swain, in press). However, in *Niveoscincus microlepidotus* embryonic nutrition is predominantly lecithotrophic (dry egg: dry neonate ratio of 0.755; Atkins et al., '06 - Ch. 3) and the egg: neonate ratio is similar to the mean ratio for other species with simple placentae (0.78 ± 0.03 , range 0.69 – 0.84) (Thompson et al., '99) in which the embryos are nourished almost entirely by yolk. By the end of autumn, the fully developed embryos have utilised nearly all their yolk store (Girling et al., '02a; '02b). Presumably, then, some of that lecithotrophic nutrition is transferred into the embryonic fat bodies that support the embryos *in utero* during the protracted later stages of gestation.

In the annually reproducing *N. metallicus*, short-term deferral of parturition by placing females in cold conditions for three weeks similarly results in significantly reduced neonatal energy reserves (Swain and Jones, '00a), inferring a trade-off between neonatal condition and date of parturition. In contrast to our results, Girling et al. ('02a) found no significant changes in fat body mass in embryos of *N. microlepidotus* collected through winter by monthly dissections of females kept in artificial hibernation in the laboratory. Again, this may reflect the differences between a relatively benign “winter” (constant 4 °C), which reflects the low energy requirements of ectotherms held at a constant temperature, and the more extreme

environment experienced by these alpine lizards in the wild (mean daily maximum: 2.7 °C; mean daily minimum: -1.4 °C for winter; Bureau of Meteorology). In the biennially reproducing gecko *H. maculatus*, there was no significant reduction in embryonic abdominal fat bodies between neonates produced by AVT injection in autumn and spring (Rock, '05): the author suggested that either this species' embryos do not rely on these lipids to survive the winter, or that components of metabolised yolk are redistributed to the embryonic fat bodies, as we have suggested for our species.

While many studies have demonstrated life history trade-offs between offspring phenotype and offspring number or maternal quality (e.g. Sinervo and Svensson, '98; Badyaev and Ghalambor, '01; Ardia, '05), this study demonstrates that such trade-offs may involve more subtle variations in offspring quality. In *N. microlepidotus*, in which embryos are fully developed in autumn, delaying birth of neonates until the spring has both costs and benefits. Enhanced survival (Olsson and Shine, '98) and size at birth may be traded-off against decreases in neonatal energy reserves, condition (in some years), growth rate and sprint speed. Contrary to our results, extended gestation of *H. maculatus* over winter results in no change in neonatal traits measured (e.g. size or energy stores), but there are no data for that species on differential mortality or growth rates for neonates born in different seasons (Rock, '05). At the finer temporal scale, the ability of female *N. microlepidotus* to delay birth if they experience adverse environmental conditions in spring does not mean they incur additional costs in terms of neonatal quality: gestating females subject to extended cold conditions in spring do defer parturition, but with no significant effects on key offspring characteristics. Therefore, at this scale our hypothesis is not supported. As in the annually reproducing *N. ocellatus*

(Atkins et al., '06 - Ch. 3), maternal decisions regarding the precise timing of parturition in spring ensure that neonates of *N. microlepidotus* are born into a benign environment without compromising offspring quality. This study provides further evidence that an ability to defer parturition represents a significant advantage for viviparous lizards living in cold climates and provides further support for the maternal manipulation hypothesis (Shine, '95; Webb et al., '06).

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LITERATURE CITED

- Ardia DR. 2005. Individual quality mediates trade-offs between reproductive effort and immune function in tree swallows. *J Anim Ecol* 74(3):517-524.
- Arlettaz R, Christe P, Lugon A, Perrin N, Vogel P. 2001. Food availability dictates the timing of parturition in insectivorous mouse-eared bats. *Oikos* 95(1):105-111.

- Atkins N, Swain R, Jones SM. 2006. Does date of birth or a capacity for facultative placentotrophy influence offspring quality in a viviparous skink, *Niveoscincus microlepidotus*? Aust J Zool 54(5):369-374.
- Atkins N, Swain R, Wapstra, E, Jones SM. in press. Late stage deferral of parturition in the viviparous lizard *Niveoscincus ocellatus* (Gray 1845): implications for offspring quality and survival. Biol J Linn Soc.
- Atkins N, Wapstra E. 2004. Successful treatment of a mite infestation in gravid spotted snow skinks (*Niveoscincus ocellatus*). Herpetofauna 34(2):66-69.
- Badyaev AV, Ghalambor CK. 2001. Evolution of life histories along elevational gradients: trade-off between parental care and fecundity. Ecology 82(10):2948-2960.
- Bernado J. 1996. Maternal effects in animal ecology. Am Zool 36:83-105.
- Blem CR, Blem LB. 1995. The eastern cottonmouth (*Agkistrodon piscivorus*) at the northern edge of its range. J Herpetol 29(3):391-398.
- Chapple DG, McCoull CJ, Swain R. 2004. Effect of tail loss on sprint speed and growth in newborn skinks, *Niveoscincus metallicus*. J Herpetol 38(1):137-140.
- Civantos E, Salvador A, Veiga JP. 1999. Body size and microhabitat affect winter survival of hatchling *Psammodromus algirus* lizards. Copeia 1999(4):1112-1117.
- Cree A, Guillette LJ Jr. 1995. Biennial reproduction with a fourteen-month pregnancy in the gecko *Hoplodactylus maculatus* from southern New Zealand. J Herpetol 29(2):163-173.
- Cumming GS, Bernard RTF. 1997. Rainfall, food abundance and timing of parturition in African bats. Oecologia 111(3):309-317.

-
- Ferguson GW, Fox SF. 1984. Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. *Evolution* 38(2):342-349.
- Fox SF. 1978. Natural selection on behavioural phenotypes of the lizard *Uta stansburiana*. *Ecology* 59(4):834-847.
- Girling JE, Jones SM, Swain R. 2002a. Delayed ovulation and parturition in a viviparous alpine lizard (*Niveoscincus microlepidotus*): morphological data and plasma steroid concentrations. *Reprod Fertil Dev* 14(1):43-53.
- Girling JE, Jones SM, Swain R. 2002b. Induction of parturition in snow skinks: can low temperatures inhibit the actions of AVT? *J Exp Zool* 293:525-531.
- Hutchinson MN, Robertson P, Rawlinson PA. 1989. Redescription and ecology of the two endemic Tasmanian scincid lizards *Leiolopisma microlepidotum* and *L. pretiosum*. *Pap Proc R Soc Tas* 123:257-274.
- Jones SM, Swain R. 1996. Annual reproductive cycle and annual cycles of reproductive hormones in plasma of female *Niveoscincus metallicus* from Tasmania. *J Herpetol* 30(2):140-146.
- Jones SM, Swain R. in press. Placental transfer of 3H-oleic acid in three species of viviparous lizards: a route for supplementation of embryonic fat bodies? *Herpetol Monogr*.
- Melville J. 1998. The evolution of locomotory mode in the lizard genus *Niveoscincus*: an ecomorphological analysis of ecology, behaviour, morphology and performance ability. Ph.D Thesis, University of Tasmania, Hobart, TAS.

-
- Olsson M, Shine R. 1996. Does reproductive success increase with age or with size in species with indeterminate growth? A case study using sand lizards (*Lacerta agilis*). *Oecologia* 105:175-178.
- Olsson M, Shine R. 1997. The seasonal timing of oviposition in sand lizards (*Lacerta agilis*): why early clutches are better. *J Exp Zool* 10:369-381.
- Olsson M, Shine R. 1998. Timing of parturition as a maternal care tactic in an alpine lizard species. *Evolution* 52(6):1861-1864.
- Olsson M, Shine R. 1999. Plasticity of frequency of reproduction in an alpine lizard, *Niveoscincus microlepidotus*. *Copeia* 1999(3):794-796.
- Rachlow JL, Bowyer RT. 1994. Variability in maternal-behavior by Dalls Sheep - environmental tracking or adaptive strategy. *J Mammal* 75(2):328-337.
- Reale D, McAdam AG, Boutin S, Berteaux D. 2003. Genetic and plastic responses of a northern mammal to climate change. *Proc R Soc Lond Ser B-Biol Sci* 270(1515):591-596.
- Rock J. 2005. Delayed parturition: constraint or coping mechanism in a viviparous gekkonid? *J Zool, Lond* 268(4):355-360.
- Shine R. 1995. A new hypothesis for the evolution of viviparity in reptiles. *Am Nat* 145:809-823.
- Shine R, Olsson M. 2003. When to be born? Prolonged pregnancy or incubation enhances locomotor performance in neonatal lizards (Scincidae). *J Evol Biol* 16:823-832.
- Sinervo B, Doughty P. 1996. Interactive effects of offspring size and timing of reproduction on offspring reproduction: experimental, maternal, and quantitative genetic aspects. *Evolution* 50(3):1314-1327.

-
- Sinervo B, Svensson E. 1998. Mechanistic and selective causes of life history trade-offs and plasticity. *Oikos* 83(3):432-442.
- Swain R, Jones SM. 2000a. Facultative placentotrophy: half-way house or strategic solution? *Comp Biochem Physiol* 127A:441-451.
- Swain R, Jones SM. 2000b. Maternal effects associated with gestation conditions in a viviparous lizard, *Niveoscincus metallicus*. *Herpetol Monogr* 14:432-440.
- Thompson MB, Speake BK, Stewart JR, Russell K, McCartney RJ, Surai PF. 1999. Placental nutrition in the viviparous lizard *Niveoscincus metallicus*: the influence of placental type. *J Exp Biol* 202:2985-2997.
- Vial JL, Stewart JR. 1985. The reproductive cycle of *Barisia monticola*: a unique variation among viviparous lizards. *Herpetologica* 41(1):51-57.
- Wapstra E, Swain R, Jones SM, O'Reilly J. 1999. Geographic and annual variation in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Aus J Zool* 47:539-550.
- Webb JK, Shine R, Christian KA. 2006. The adaptive significance of reptilian viviparity in the tropics: testing the maternal manipulation hypothesis. *Evolution* 60(1):115-122.

Chapter 3

Does date of birth or a capacity for facultative placentotrophy influence offspring quality in a viviparous skink, *Niveoscincus microlepidotus*?

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ABSTRACT

Niveoscincus microlepidotus is a viviparous lizard that exhibits biennial reproduction: births in spring optimise offspring survival in this species' alpine habitat. Over three annual activity seasons, we investigated the hypothesis that date of birth influences offspring characteristics. In the first year of our study, there was no effect of date of birth on any offspring characteristic measured. In the second year, later born offspring grew significantly faster. In the third year, later born offspring were significantly longer and heavier at birth. These varying results may be attributable to differences in basking opportunities for the gestating females due to annual variations in spring weather patterns.

To test the hypothesis that both lecithotrophic (yolk) and placentotrophic nutrients support embryos during their extended gestation, we determined the degree of placentotrophy for this species. The mean dry neonate: dry egg ratio of 0.755 indicates that this species is predominantly lecithotrophic. Only in the first year of the study did the data suggest a potential for facultative placentotrophy in this

species. We therefore suggest that in *N. microlepidotus* lecithotrophic nutrition is converted into embryonic fat bodies that support the embryo *in utero* over winter until spring parturition.

INTRODUCTION

In reptiles, oviposition (egg-laying) date, hatching date and birth date may affect both the probability of offspring survival and offspring phenotype (Blem and Blem 1995; Olsson and Shine 1996; Sinervo and Doughty 1996; Olsson and Shine 1997; Civantos *et al.* 1999; Shine and Olsson 2003). In viviparous species (live bearers), experimental manipulation of maternal access to basking results in an increased range of dates over which neonates are born, and significant variations in offspring phenotype (Shine and Harlow 1993; Mathies and Andrews 1997; Doughty and Shine 1998; Shine and Downes 1999; Swain and Jones 2000b; Wapstra 2000).

Niveoscincus microlepidotus is a viviparous alpine skink with an unusual biennial reproductive cycle (Hutchinson *et al.* 1989; Olsson and Shine 1998; 1999): ovulation occurs in spring, and embryonic development continues over summer. By late autumn, the embryos are fully formed, and have used nearly all of their yolk (Girling *et al.* 2002a; 2002b), but parturition is delayed until late in the following spring so that gestation takes approximately a year to complete. This represents an extraordinarily long interval between the completion of embryonic development and parturition. Biennial reproduction presumably represents a significant and necessary trade-off of neonatal quality at birth versus their probability of survival (Olsson and Shine 1998): in particular, compared with those of fully developed embryos in

autumn, embryonic fat bodies are significantly depleted in embryos collected just before parturition (Atkins *et al.* unpublished data – Ch. 2).

Even after emergence in spring these females may trade off offspring quality against the probability of those offspring's survival after birth. Parturition does not occur immediately after spring emergence: the young may not be born until several weeks later (Olsson and Shine 1999). What consequences, then, might parturition date have for the quality of the neonates? In this study, we aim to investigate the relationships between date of birth and a suite of offspring characteristics in *N. microlepidotus* to test the hypothesis that deferral of parturition represents a cost in terms of offspring fitness that is outweighed by the benefits of being born into a more benign environment, later in spring. Shine and Olsson (2003) investigated the effects of parturition date on survival, offspring mass and sprint speed at birth in *N. microlepidotus* and found that offspring mass decreased with parturition date, while sprint speed and the probability of survival increased. To test our hypothesis, a more comprehensive analysis of the influence of birth date on a fuller suite of key offspring characteristics, including neonatal fat reserves and growth, is required.

Does prolonging embryonic survival *in utero* for what may be several weeks also incur significant additional energetic costs for the offspring? Supporting embryos through this protracted gestation must also result in energetic costs to the mother. Swain and Jones (2000a) have previously suggested that embryos of viviparous lizards utilise their own fat reserves (abdominal fat bodies) to survive if parturition is delayed after the completion of embryonic development. Furthermore, they argue that facultative placental transfer of nutrients (Stewart 1989) supplements those fat bodies. In our study we also investigate the hypothesis that females of *N. microlepidotus* may utilise facultative placentotrophy to supplement embryonic

nutrition. If yolk stores are significantly depleted even before winter (Girling *et al.* 2002a; Girling *et al.* 2002b), are these embryos supported by different mechanisms, especially near the end of this protracted gestation? *Niveoscincus microlepidotus* appears to have a simple Type II placenta (J. Stewart, personal communication; Weekes 1935) and ovulates a large yolky egg. We aim first, to determine the degree of placentotrophy (i.e. the proportion of organic nutrients not supplied by the yolk), which may be assessed by a simple comparison of egg dry weight and neonate dry weight (Stewart and Thompson 2000). Second, we aim to determine if there may be a facultative component to placental contributions to embryonic nutrition in *N. microlepidotus*. Stewart (1989) considered that a necessary criterion for demonstrating facultative placentotrophy is that larger females produce larger neonates independent of egg mass: we use this premise as the basis of our investigation.

METHODS

Female *Niveoscincus microlepidotus* were collected by hand, noosing or mealworm ‘fishing’ from near the summit (1270 m) of Mt. Wellington, Hobart, Tasmania, Australia (42°53’S, 147°14’E). As this species exhibits a biennial reproductive cycle, females in one of two reproductive conditions are present in the population at any one time, so reproductive status was determined by gentle palpation (Girling *et al.* 2002a). Pregnant females near parturition were collected in October of 2002 (N = 30), 2003 (N = 40), and 2004 (N = 20) and brought into the laboratory. All animals brought into the laboratory were treated for mites by dipping in Neguvon[®], with further regular treatments as suggested by Atkins and Wapstra

(2004 - App. 1). Lizards were maintained in pairs in plastic containers (30 cm x 20 cm x 10 cm) in an air-conditioned room provided with a 11:13 h light: dark regime at natural light intensities and a background temperature of 12 °C (standard laboratory conditions). Each container included a basking platform and a layer of paper pellets, and a thermal gradient (12 – 35 °C) was obtained through a low wattage light bulb positioned over one end of the cage (light on: 09:00 – 17:00). Containers were repositioned within the room several times weekly to minimise position effects. The adult lizards were fed with *Tenebrio* larvae (mealworms), meaty cat food or pear-banana baby food three times per week. A calcium supplement was dusted over the food and multi-vitamin supplements were supplied in the drinking water, which was available *ad libitum*.

Containers were checked for births several times per day. The day that the first female gave birth was designated day 1, and for subsequent births, the date of birth was recorded as days since day 1. Maternal size (snout-vent length - SVL) and mass (g) were recorded post-partum.

Offspring maintenance and evaluation of fitness

For litters of two or more neonates (litter size \pm SE: 2.5 ± 0.15 , range: 1-4), one neonate was killed on the day of birth. Its mass and SVL recorded, and the abdominal fat bodies removed by dissection. The carcass of the neonate and its fat bodies were dried in an oven at ~ 70 °C for 5 days, and their dry weights determined.

Birth mass, SVL, tail length, and sprint speed were measured for all other neonates. A measure of body condition was obtained by generating the Student residuals from a mass - SVL regression (using data from all neonates). Sprint speed was measured using a temperature-controlled (26 °C) computerised racetrack with

three sets of photoelectric detectors. Animals were first warmed to 26 °C for 30 min (Melville 1998) and then induced to run by teasing with a soft paintbrush. Each neonate was run twice, with no rest between runs, down the 1 m track. Two times were obtained from each run, with the fastest speed (cm/s) of the four being used for analysis.

In 2002 and 2003, post-natal growth rate and basking frequency were also assessed for all neonates. Neonates were individually marked with non-toxic silver paint and allocated randomly to groups of five, which were kept under the standard laboratory conditions (as described above) for 11 weeks after birth. To minimise position effects, containers were repositioned each time the lizards were fed. Neonates were fed three times per week with a variety of meaty cat food, pear-banana baby food and nematodes (white worms). All lizards were periodically given a calcium supplement in their food; multi-vitamin supplements were available in the drinking water, which was available *ad libitum*. Dominance hierarchies were not established within these groups, and animals shared the basking site with no apparent conflict. Mass, lengths (SVL and tail) and sprint speed were measured every second week for 11 weeks and neonates that did not survive this entire period were excluded from the analyses. During this period the growth parameters increased linearly: consequently, the slopes of the linear regressions of mass (or either of the length measurements) against time were taken as indices of growth (Swain and Jones 2000b). Basking frequency was assessed in alternate weeks by rapid surveying of cages at 15 min intervals throughout the day between 10 am and 4 pm (one day a fortnight), and noting which animals were basking (total observations: 25). At the end of the 11 weeks, neonates were released at the site of maternal capture.

Collection of preovulatory eggs and calculation of the degree of placentotrophy

Vitellogenic females were collected from the same site as the pregnant females in October 2002 (N = 13), and 2003 (N = 9), as close as possible to the time of ovulation (Olsson and Shine 1999). Females were weighed, measured (SVL) and then killed by chilling and rapid decapitation and the oviducts removed by dissection. Eggs were counted, and removed from the oviducts for measurement of egg size (diameter) and egg wet and dry weight (samples were dried in an oven at ~ 70 °C for approximately 2 days).

The degree of placentotrophy was estimated by calculating the ratio of the dry mass of neonates at birth relative to the dry mass of preovulatory eggs. As this is a biennially reproducing species, egg data were compared with data for neonates collected the following year. To assess the capacity for facultative placentotrophy, the relationship of egg size to maternal size was compared to the relationship of mean neonate mass (at birth in spring) to maternal mass (Stewart 1989; Swain and Jones 2000a).

Statistics

All statistical analyses were carried out in SAS (v 6.12). To avoid the problems of non-independence of data derived from siblings, we carried out all our analyses on litter means. Regression analyses were conducted to determine whether date of birth and neonatal measures were correlated, and to determine relationships between maternal and neonatal mass, in each year that data were collected.

Neonates that were found at birth with part of their tail missing were excluded from analyses including birth weight or tail length. When tail loss occurred after the day of birth, data for growth in mass and tail growth were excluded but not

data for SVL: in *N. metallicus*, caudal autotomy at birth does not inhibit growth measured as an increase in SVL (Chapple *et al.* 2004).

RESULTS

There was a significant effect of date of birth on some offspring characteristics, but in only two of the three years of the study. In the first year of the study (2002), births were spread over 35 days and there was no effect of birth date on any characteristic of the neonates ($P > 0.05$, Table 1). In the following year (2003), births were spread over 24 days and there was a significant effect of birth date on growth rate (change in mass) of the neonates (Regression, $F_{(1,21)} = 6.536$, $P = 0.0184$, $r^2 = 0.2374$), with a greater growth rate associated with a later date of birth (Figure 1). However, there were no significant effects of date of birth on any other offspring characteristics ($P > 0.05$). In 2004, although births were spread over only 12 days, there were significant effects of date of birth on several key offspring characteristics and performance criteria. There was a significant effect of date of birth on wet mass at birth (Regression, $F_{(1,18)} = 6.352$, $P = 0.02$, $r^2 = 0.2608$) (Figure 2A), dry mass at birth (Regression, $F_{(1,16)} = 7.063$, $P = 0.02$, $r^2 = 0.3063$) (Figure 2B), and SVL at birth (Regression, $F_{(1,18)} = 7.97$, $P = 0.01$, $r^2 = 0.3069$) (Figure 2C), with neonates being both heavier and longer (SVL) with increasing date of birth. All other characteristics (tail length, fat bodies, neonate condition and sprint speed) showed no significant relationship with date of birth ($P > 0.05$).

Table 1. *Niveoscincus microlepidotus* offspring characteristics at and after birth in 2002

Values are means for each litter \pm SE (N).

Mass - wet (mg)	319.1 \pm 8.4 (28)
Mass - dry (mg)	64.9 \pm 2.4 (22)
SVL (mm)	26.1 \pm 0.2 (29)
Tail length (mm)	35.5 \pm 0.4 (27)
Fat bodies - dry (mg)	2.1 \pm 0.4 (22)
Neonate condition (residuals)	-0.6 \pm 0.3 (28)
Sprint speed day 1 (cm/s)	16.6 \pm 0.8 (26)
Growth (mg/day)	5.4 \pm 0.4 (26)
Growth - SVL (mm/day)	0.1 \pm 0.004 (27)
Growth - tail length (mm/day)	0.1 \pm 0.007 (25)
Sprint speed day 1-63 (cm/s)	15.8 \pm 0.6 (27)
Basking intensity (obs/day)	16.1 \pm 0.7 (27)

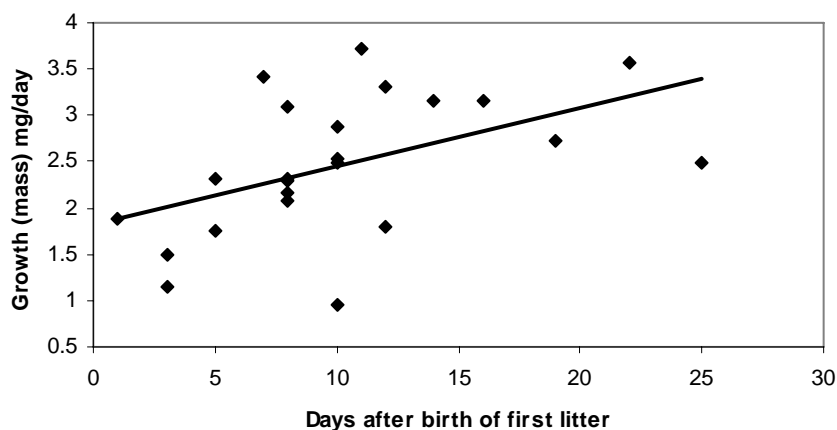
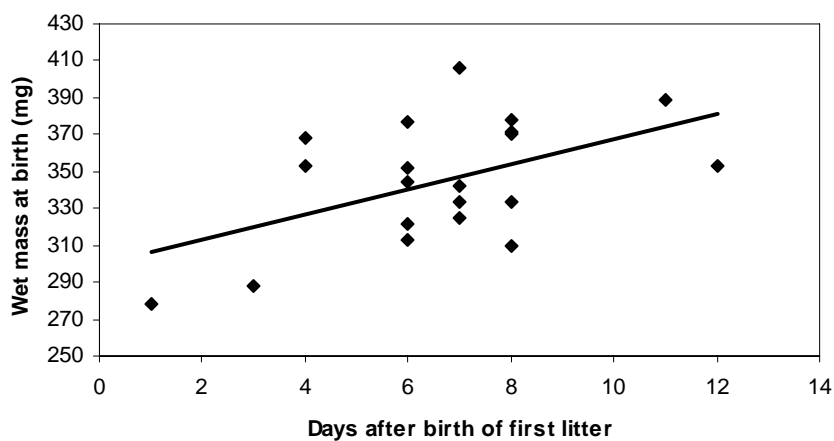
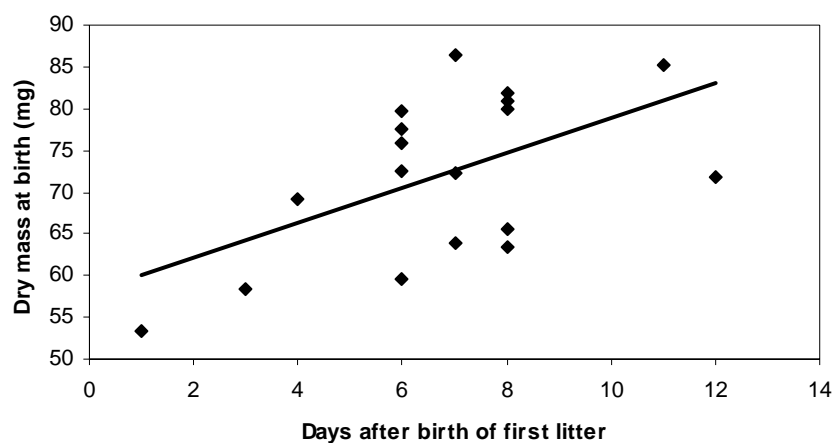


Figure 1. Relationship between birth date and growth rate (mass: mg/day) of *Niveoscincus microlepidotus* neonates born in 2003. Day 1 represents the first birth in that experimental population. Each point represents the mean growth rate for a single litter.

A



B



C

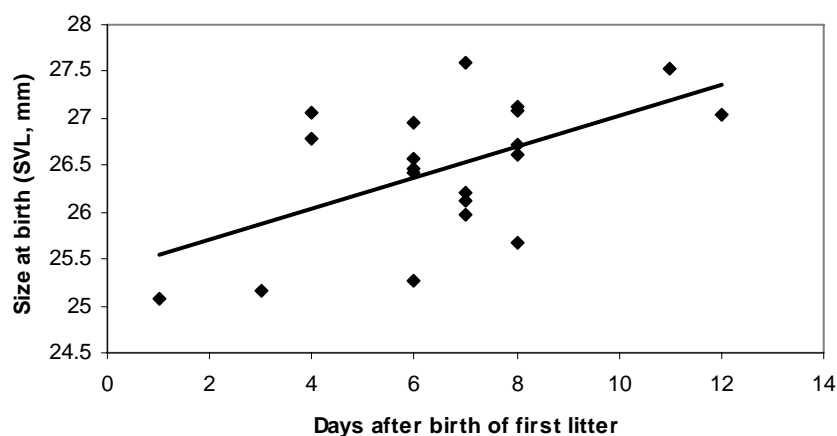


Figure 2. Relationship between birth date and neonatal characteristics at birth (A: wet mass; B: dry mass; C: SVL) for young of *Niveoscincus microlepidotus* born in 2004. Day 1 represents the first birth in that experimental population. Each point represents the mean mass or size for a single litter.

*Degree of placentotrophy (neonate: egg ratio) and evidence for facultative
placentotrophy*

The neonate: egg ratios calculated using data from eggs and neonates collected in 2003 and 2004 were 0.76 and 0.75 respectively (Table 2). Analysis of data collected in both 2002 and 2003 showed that in *N. microlepidotus* the size and mass (wet and dry) of preovulatory eggs did not correlate with maternal SVL or maternal mass ($P > 0.05$). The 2002 and 2003 data for the size of eggs were combined ($P > 0.05$) and their relationship with maternal size (SVL) is presented in Figure 3. In neither 2003 nor 2004 was there a significant relationship between maternal mass and neonatal mass ($P > 0.05$, Figure 4B, 4C). However, analysis of data collected in 2002 showed that in that year larger females did give birth to larger neonates ($F_{(1,20)} = 13.880$, $P = 0.001$, $r^2 = 0.410$, Figure 4A).

Table 2. Data for mean dry mass of pre-ovulatory eggs and neonatal mass in *Niveoscincus microlepidotus*

Values are means for each litter \pm SE (N).

Dry mass of neonate	Dry mass of eggs	Ratio of neonate: egg
2003 - 69.3 \pm 1.7 mg (36)	2002 - 91.8 \pm 3.8 mg (13)	0.76
2004 - 72.1 \pm 2.3 mg (18)	2003 - 95.6 \pm 3.1 mg (9)	0.75

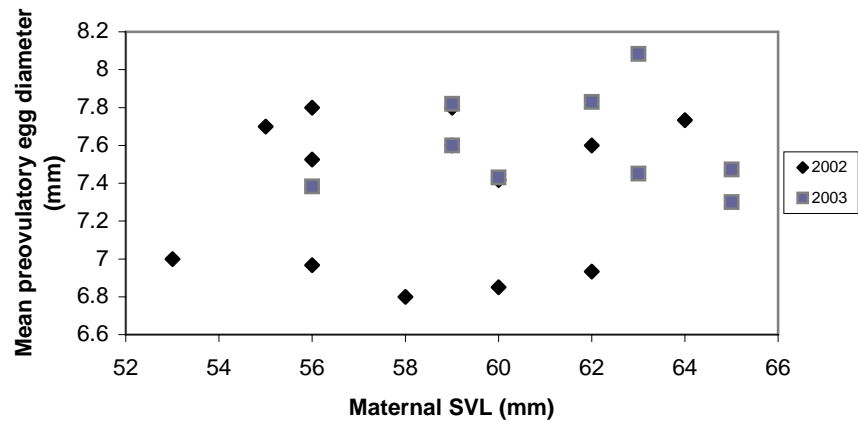
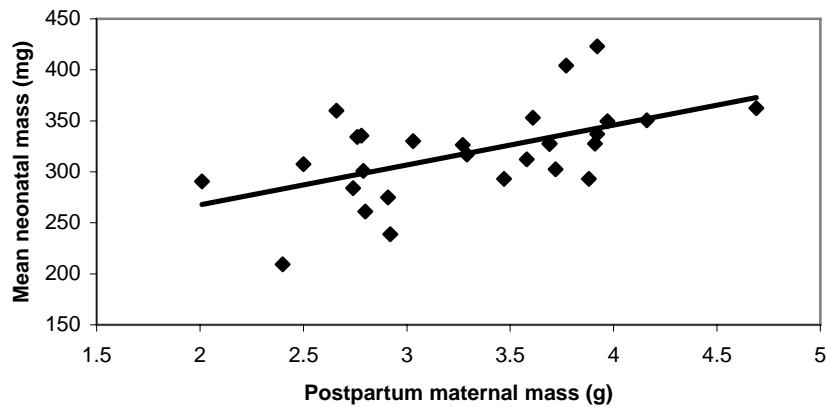
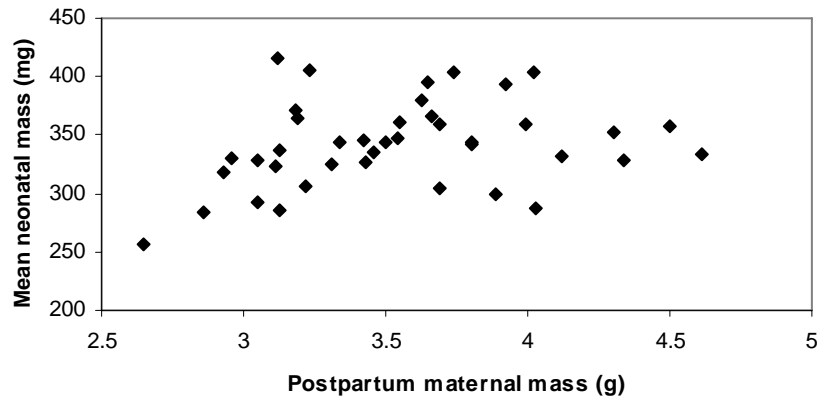


Figure 3. Relationship between preovulatory egg diameter and maternal size (SVL) in *Niveoscincus microlepidotus* for animals collected in 2002 and 2003. Values are litter means.

A



B



C

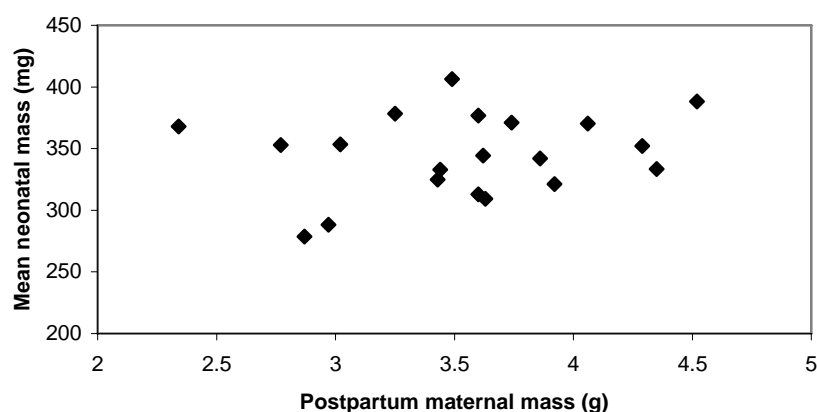


Figure 4. Relationship between neonate mass and postpartum maternal mass in *Niveoscincus microlepidotus* collected in A) 2002, B) 2003, and C) 2004. Values are litter means.

DISCUSSION

In *Niveoscincus microlepidotus*, neonatal characteristics show no consistently strong correlations with date of birth. For neonates born in 2003, only growth (mass) was correlated with date of birth, while several measures of offspring fitness were correlated with date of birth for young born in 2004, the year with the smallest spread of births in the laboratory. Where we did detect an effect, later-born offspring were advantaged compared with earlier-born offspring: in lizards, offspring size is often an indicator of quality (Bernado 1996) and a strong indicator of the probability of survival (e.g. Fox 1978; Ferguson and Fox 1984; Sinervo and Doughty 1996; Civantos *et al.* 1999). It appears that in *N. microlepidotus* any effect of date of birth on neonatal characteristics is variable between years, and the direction of that effect may also vary. In an earlier study of *N. microlepidotus* (Shine and Olsson 2003) that

examined only certain measures of offspring quality, offspring mass decreased with parturition date in two of the three years of the study, while mean sprint speed increased with date of birth in the two years that it was measured. These apparently contradictory results may reflect responses to yearly variation in spring weather patterns, which would have profound effects on the activity patterns of these alpine lizards. Crucially, variation in spring weather would affect the basking opportunities available to gestating females, which has been shown to affect offspring phenotype in several species of viviparous lizards (Shine and Harlow 1993; Shine and Downes 1999; Swain and Jones 2000b; Wapstra 2000), while in *Uta stansburiana*, local environmental effects may alter current reproductive investment in any given season (Sinervo and DeNardo 1996).

There was no effect of birth date on either neonate condition or fat reserves in any of the three data sets, suggesting that a later parturition date does not incur significant energetic costs for these late-stage embryos. Does this mean that in this species supplementation of embryonic fat bodies by facultative placentotrophy is not important in this species? In *Niveoscincus microlepidotus* embryonic nutrition is predominantly lecithotrophic: the neonate: egg ratio we measured is similar to the mean ratio (0.78 ± 0.03 , range 0.69 – 0.84) for other species with simple placentae that rely primarily upon yolk to support their embryos (Thompson *et al.* 1999a). Using Stewart (1989)'s criterion, we were able to demonstrate a potential for facultative placentotrophy only for data collected in the first of the three years of our study. Facultative placentotrophy has been documented in the predominantly lecithotrophic *Virginia striatula* (Stewart 1989), *Thamnophis ordinoides* (Stewart *et al.* 1990), and *N. metallicus* (Thompson *et al.* 1999a; Swain and Jones 2000a), and in the more placentotrophic *Pseudemoia spenceri* (Thompson *et al.* 1999c) and *P.*

pagenstecheri (Thompson *et al.* 1999b). In all but one of these studies (Thompson *et al.* 1999c), the females were maintained in the laboratory for the entire duration of gestation: laboratory conditions that provide optimum food availability and basking opportunities may ensure that such females are able to provide facultative supplementation to yolk supplies. Although we have experimentally demonstrated that placental transfer of lipid (^3H -oleic acid) into embryos and embryonic abdominal fat bodies in females of *N. microlepidotus* does occur (Jones and Swain unpublished data), our observations here suggest that variation in food availability and/or environmental conditions in this species' alpine environment may preclude detectable facultative supplementation of yolk supplies in some, but not all, seasons. Several other studies have indicated that the degree of placentotrophy may vary both within species (Stewart 1989; Thompson *et al.* 1999a; 2000) and between years (Sangha *et al.* 1996), or may represent transfer only of specific embryonic requirements (eg. calcium in *Thamnophis ordinoides*: Stewart *et al.* 1990). It is possible therefore that our observations reflect a potential capacity for facultative placental transfer of nutrients that is rarely possible in the harsh natural environment experienced by our alpine species.

Thus our prediction that *N. microlepidotus* uses facultative placentotrophy to enhance embryonic energy stores was not supported. However, by the end of autumn, the fully developed embryos have utilised nearly all their yolk store (Girling *et al.* 2002a; 2002b). Presumably, then, some of that yolk is converted into embryonic fat bodies that support the embryos *in utero* over winter. Indeed, we have demonstrated that embryonic fat reserves decrease significantly over winter in this species (Atkins *et al.* unpublished data – Ch 2). We therefore suggest that fat stores which support the embryos through this extraordinarily extended gestation are

derived from lecithotrophic energy stores, and that a potential capacity for facultative placentotrophy may provide an additional advantage to offspring born in some years only.

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REFERENCES

Atkins, N., and Wapstra, E. (2004). Successful treatment of a mite infestation in gravid spotted snow skinks (*Niveoscincus ocellatus*). *Herpetofauna* **34**, 66-69.

Bernado, J. (1996). Maternal effects in animal ecology. *American Zoologist* **36**, 83-105.

Blem, C.R., and Blem, L.B. (1995). The eastern cottonmouth (*Agkistrodon piscivorus*) at the northern edge of its range. *Journal of Herpetology* **29**, 391-398.

Chapple, D.G., McCoull, C.J. and Swain, R. (2004). Effect of tail loss on sprint speed and growth in newborn skinks, *Niveoscincus metallicus*. *Journal of Herpetology* **38**, 137-140.

Civantos, E., Salvador, A. and Veiga, J.P. (1999). Body size and microhabitat affect winter survival of hatchling *Psammodromus algirus* lizards. *Copeia* **1999**, 1112-1117.

Doughty, P., and Shine, R. (1998). Reproductive energy allocation and long-term energy stores in a viviparous lizard (*Eulamprus tympanum*). *Ecology* **79**, 1073-1083.

Ferguson, G.W., and Fox, S.F. (1984). Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. *Evolution* **38**, 342-349.

Fox, S.F. (1978). Natural selection on behavioural phenotypes of the lizards *Uta stansburiana*. *Ecology* **59**, 834-847.

Girling, J.E., Jones, S.M. and Swain, R. (2002a). Delayed ovulation and parturition in a viviparous alpine lizard (*Niveoscincus microlepidotus*): morphological data and plasma steroid concentrations. *Reproduction Fertility and Development* **14**, 43-53.

Girling, J.E., Jones, S.M. and Swain, R. (2002b). Induction of parturition in snow skinks: Can low temperatures inhibit the actions of AVT? *Journal of Experimental Zoology* **293**, 525-531.

Hutchinson, M.N., Robertson, P. and Rawlinson, P.A. (1989). Redescription and ecology of the two endemic Tasmanian scincid lizards *Leiopisma microlepidotum* and *L. pretiosum*. *Papers and Proceedings of the Royal Society of Tasmania* **123**, 257-274.

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.

Melville, J. (1998) The evolution of locomotory mode in the lizard genus *Niveoscincus*: An ecomorphological analysis of ecology, behaviour, morphology and performance ability. Ph.D. Thesis, University of Tasmania, Hobart.

Olsson, M., and Shine, R. (1996). Does reproductive success increase with age or with size in species with indeterminate growth? A case study using sand lizards (*Lacerta agilis*). *Oecologia* **105**, 175-178.

Olsson, M., and Shine, R. (1997). The seasonal timing of oviposition in sand lizards (*Lacerta agilis*): why early clutches are better. *Journal of Experimental Zoology* **10**, 369-381.

Olsson, M., and Shine, R. (1998). Timing of parturition as a maternal care tactic in an alpine lizard species. *Evolution* **52**, 1861-1864.

Olsson, M., and Shine, R. (1999). Plasticity of frequency of reproduction in an alpine lizard, *Niveoscincus microlepidotus*. *Copeia* **1999**, 794-796.

Sangha, S., Smola, M.A., McKinney, S.L., Crotzer, D.R., Shadrix, C.A. and Stewart, J.R. (1996). The effect of surgical removal of oviductal eggs on placental function and size of neonates in the viviparous snake *Virginia striatula*. *Herpetologica* **52**, 32-36.

Shine, R., and Downes, S.J. (1999). Can pregnant lizards adjust their offspring phenotypes to environmental conditions? *Oecologia* **119**, 1-8.

Shine, R., and Harlow, P. (1993). Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia* **96**, 122-127.

Shine, R., and Olsson, M. (2003). When to be born? Prolonged pregnancy or incubation enhances locomotor performance in neonatal lizards (Scincidae). *Journal of Evolutionary Biology* **16**, 823-832.

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.

Sinervo, B., and Doughty, P. (1996). Interactive effects of offspring size and timing of reproduction on offspring reproduction: experimental, maternal, and quantitative genetic aspects. *Evolution* **50**, 1314-1327.

Stewart, J.R. (1989). Facultative placentotrophy and the evolution of squamate placentation: quality of eggs and neonates in *Virginia striatula*. *The American Naturalist* **133**, 111-137.

Stewart, J.R., Blackburn, D.G. and Hoffman, L.H. (1990). Nutritional provision to embryos in a predominantly lecithotrophic placental reptile, *Thamnophis ordinoides* (Squamata: Serpentes). *Physiological Zoology* **63**, 722-734.

Stewart, J.R., and Thompson, M.B. (2000). Evolution of placentation among squamate reptiles: recent research and future directions. *Comparative Biochemistry & Physiology Part A* **127**, 411-431.

Swain, R., and Jones, S.M. (2000a). Facultative placentotrophy: half-way house or strategic solution? *Comparative Biochemistry and Physiology Part A* **127**, 441-451.

Swain, R., and Jones, S.M. (2000b). Maternal effects associated with gestation conditions in a viviparous lizard, *Niveoscincus metallicus*. *Herpetological Monographs* **14**, 432-440.

Thompson, M.B., Speake, B.K., Stewart, J.R., Russell, K., McCartney, R.J. and Surai, P.F. (1999a). Placental nutrition in the viviparous lizard *Niveoscincus metallicus*: the influence of placental type. *Journal of Experimental Biology* **202**, 2985-2997.

Thompson, M.B., Stewart, J.R. and Speake, B.K. (2000). Comparison of nutrient transport across the placenta of lizards differing in placental complexity.

Comparative Biochemistry and Physiology Part A **127**, 469-479.

Thompson, M.B., Stewart, J.R., Speake, B.K., Russell, K., McCartney, R.J. and Surai, P.F. (1999b). Placental nutrition in a viviparous lizard (*Pseudemoia pagenstecheri*) with a complex placenta. *Journal of Zoology, (London)* **248**, 295-305.

Thompson, M.B., Stewart, J.R., Speake, B.K., Russell, K.J. and McCartney, R.J. (1999c). Placental transfer of nutrients during gestation in the viviparous lizard, *Pseudemoia spenceri*. *Journal of Comparative Physiology B* **169**, 319-328.

Wapstra, E. (2000). Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Functional Ecology* **14**, 345-353.

Weekes, H.C. (1935). A review of placentation among reptiles with particular regard to the function and evolution of the placenta. *Proceedings of the Zoological Society of London* **3**, 625-645.

Chapter 4

Late stage deferral of parturition in the viviparous lizard *Niveoscincus ocellatus* (Gray 1845): implications for offspring quality and survival

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ABSTRACT

The ability of viviparous lizards to defer parturition after completion of embryonic development is a potentially key strategy for enhancing offspring fitness. Using the spotted snow skink, *Niveoscincus ocellatus*, we investigated the ability of females to defer birth if placed into cold conditions at the same time, for one, two or three weeks at the end of gestation. The ability to defer birth was compared between two populations at each end of the species' altitudinal range. We hypothesised that females from the high elevation population would be less likely to defer parturition under extended periods of poor conditions, thus ensuring that young were not born too late in the season. In all but the three week treatment group from the high elevation population, females were able to defer birth when placed under cold conditions: for all groups there was no treatment effect on offspring phenotype at birth, dispersal distance or survivorship after release. However, there was a significant negative effect of treatment on offspring growth measured after release,

with the results differing between the populations. Females from the high elevation population were less able to defer birth under long periods (three weeks) of cold conditions, and this was accompanied by a decrease in viability of the offspring.

INTRODUCTION

Phenotypic variation among offspring is attributable not only to genotypic variation but also to the environmental experience of the parents, particularly the mother (Mousseau & Fox, 1998; Qvarnstrom & Price, 2001). Prenatal effects may also influence juvenile dispersal (Massot & Clobert, 2000) with subsequent effects on survival, and avoidance of competition and/or inbreeding, and, thus, offspring fitness (e.g. Clobert *et al.*, 1994; Doughty & Sinervo, 1994; Doughty, Sinervo & Burghardt, 1994; Massot & Clobert, 1995; 2000; Olsson, Gullberg & Tegelstrom, 1996; Olsson & Shine, 2003).

In egg laying species, such as birds and many reptiles, prenatal effects are primarily environmental. In oviparous reptiles, for example, incubation temperature, which may be under maternal control through nest site selection (Shine & Harlow, 1996), affects offspring phenotype (e.g. Du, Shou & Liu, 2003; Elphick & Shine, 1998; Hare & Daugherty, 2002; Shine, Elphick & Harlow, 1997; Van Damme *et al.*, 1992), while in birds, incubation temperature is influenced both by nest site selection (Lloyd & Martin, 2004) and by the incubation behaviour of the parents (Poussart, Gauthier & Larochelle, 2001; Tarboton, 1993). In viviparous species, however, maternal effects during gestation may have important implications for offspring fitness (Reinhold, 2002). In endothermic mammals, the environment experienced by the female during gestation can affect both gestation length (Bowen *et al.*, 2003;

Reale *et al.*, 2003) and offspring fitness (Bowen *et al.*, 2003). In viviparous ectothermic species (such as many lizards and snakes), the environment experienced by the female during pregnancy can also influence both gestation length and the phenotype and, therefore, the fitness of the offspring (Bernado, 1996); furthermore, the effects of environmental temperature might be predicted to be more pronounced than in endothermic species. Indeed, in viviparous lizards, both basking opportunities and food availability for pregnant females affect the phenotype of the offspring (Massot & Clobert, 1995; Shine & Harlow, 1993; Sorci & Clobert, 1997; Swain & Jones, 2000b; Wapstra, 2000). Variations in offspring phenotype, such as a decrease in offspring mass, as a result of decreased basking opportunity are usually attributable to the increase in gestation length (a direct effect of incubation temperature) (Schwarzkopf & Shine, 1991; Shine & Downes, 1999; Shine & Harlow, 1993; Swain & Jones, 2000b; Wapstra, 2000), rather than reflecting adaptive tactics by the female (Shine & Olsson, 2003).

However, maternal influences on offspring characteristics may be adaptive if mothers can use cues about future environmental quality (Bernado, 1991; Olsson & Shine, 1998). Indeed, parturition date may be controlled by the female: in viviparous squamates, parturition may be deferred for a period of time after completion of embryonic development to ensure that neonates are born under conditions which are more favourable for offspring fitness and survival (Girling, Jones & Swain, 2002; Mathies & Andrews, 1995; Olsson & Shine, 1998; 1999; Swain & Jones, 2000a; 2000b). In extreme cases, the length of deferral is such that reproduction becomes biennial (e.g. *Niveoscincus microlepidotus* (Hutchinson, Robertson & Rawlinson, 1989; Olsson & Shine, 1998), *Hoplodactylus maculatus* (Cree & Guillette, 1995) and *Barisia monticola* (Vial & Stewart, 1985)): in these cases, embryonic development is

typically completed in autumn but parturition is delayed through hibernation until well into the following spring. Similarly, some oviparous species demonstrate the ability to retain eggs in conditions where oviposition sites or proximate factors (e.g. severe cold, lack of rains) are not congenial for oviposition (Andrews & Rose, 1994; Shanbhag, Saidapur & Radder, 2003; Shine, 1995; Warner & Andrews, 2003). For example, it has been shown that *Calotes versicolor* can retain eggs in the oviduct for at least six months before oviposition (Radder, Shanbhag & Saidapuir, 1998).

It has further been suggested that, in viviparous reptiles, retaining fully developed embryos allows additional time for differentiation and development, which can result in neonates with enhanced locomotor or sensory abilities (Shine & Olsson, 2003). However, if parturition is deferred, the embryos require sufficient energy resources to enable them to survive until birth. In both oviparous and viviparous species, there will be trade-offs in terms of offspring fitness if hatching or birth is delayed: energy stores that would have been available for neonatal maintenance or growth will be reduced, but the immediate survival of neonates may be enhanced. In viviparous lizards, those stores may be supplemented via the placenta, even in species providing substantial yolk for the embryos. Indeed it has been hypothesized that placentotrophy first evolved as a facultative mechanism (Stewart, 1989) enabling mothers to supplement an adequate yolk supply, thus enhancing offspring condition if circumstances are favourable during gestation (Swain & Jones, 2000a; 2000b; Thompson *et al.*, 1999a; Thompson *et al.*, 1999b; Thompson *et al.*, 1999c). Enhanced offspring condition is thus achieved by providing embryos with larger fat bodies which may be utilised after birth for growth or to provide an additional energy reserve if there is poor food availability; alternatively,

this “bonus” energy may be utilised before birth under conditions when parturition is delayed (Swain & Jones, 2000a).

The ability to defer parturition has been explored using the viviparous lizard species, *Niveoscincus ocellatus*, which is widespread across Tasmania, Australia, occupying rocky outcrops from sea level to sub alpine regions at about 1200 m (Wapstra & Swain, 1996; 2001). The species’ range thus includes both cool-temperate (low elevations) and cold-temperate (high elevations) zones (Rawlinson, 1974). In cool-temperate conditions, *N. ocellatus* is active from the beginning of spring (September) until late autumn (mid-May) with occasional emergence in winter; however, at high elevations, the season is reduced to approximately October to April, accompanied by an unbroken winter torpor (Wapstra *et al.*, 1999). Across its range, the species retains an annual reproductive cycle rather than the biennial cycle exhibited by its sister species *N. microlepidotus* and *N. greeni* (Hutchinson *et al.*, 1989; Olsson & Shine, 1998; 1999). There are, however, marked geographic differences in the timing of key events within the female reproductive cycle. In higher elevation populations of *N. ocellatus*, ovulation occurs approximately one month later than in populations at lower elevations, and the timing of births is offset to a similar extent (Wapstra *et al.*, 1999).

Might this variation in the timing of natural births between populations in *N. ocellatus* be reflected in differences in their ability to defer birth under adverse environmental conditions? Furthermore, could this reflect subtle differences in their strategies for optimising offspring fitness? With a reduced activity season and later parturition, offspring of high elevation populations have a shorter time period available for growth, accumulation of energy reserves, and dispersal before cessation of activity in autumn. We hypothesize that, although populations from both a high

and a low elevation locality will be able to tolerate short periods of poor weather (as often occurs in this temperate environment), the high elevation population will be less likely to defer birth under extended periods of poor conditions, to ensure that young are not born too close to winter. Temperature is perhaps the most important proximate environmental factor controlling the timing of reproduction in reptiles (Licht, 1984). Thus, we manipulated environmental conditions at the expected time of parturition by exposing gestating females from both populations to three different periods of time in cold conditions, and investigated the resultant effects on the timing of parturition and neonatal phenotype. Since the date of either oviposition or parturition has been shown to influence offspring survival in oviparous and viviparous lizards respectively (Civantos, Salvador & Veiga, 1999; Olsson & Shine, 1996; 1997; Sinervo & Doughty, 1996), we also assessed survival, growth and dispersal of offspring released into their natural populations over their first winter.

METHODS

Animal capture and husbandry

Pregnant female *Niveoscincus ocellatus* were collected, first, from a low elevation population near Orford (42°34'S, 147°52'E) on the east coast (50-75 m above sea level) of Tasmania, Australia: this site is typical of much of the coastal, cool temperate, region of Tasmania (Rawlinson, 1974). Second, the Central Plateau near Lake Augusta (41°51'S, 146°34'E) was chosen to represent a high elevation population of the species: this subalpine location lies approximately 1200 m above sea level, and is within the cold temperature region of Tasmania (Rawlinson, 1974). Further details of these populations, including reproductive and life history variations

between populations can be found in Wapstra & O'Reilly (2001), Wapstra & Swain (2001), Wapstra *et al.*, (1999), Wapstra, Swain & O'Reilly (2001). Lizards were caught using mealworm “fishing”, noosing with long rods, or by hand. They were returned to the laboratory and treated for mites by dipping in Neguvon[®], with further dippings as required (Atkins & Wapstra, 2004 - App. 1). At the time of collection, the females were about two weeks from the expected date of parturition for their respective population (Wapstra *et al.*, 1999): this was confirmed by dissection of two females from each group, both found to contain embryos at approximately stage 39 of development (Dufaure & Hubert, 1961). The females were established in pairs in plastic containers (30 cm x 20 cm x 10 cm), with mesh lids, a basking site, and paper pellets for bedding, in an air-conditioned room provided with a 11:13 h light : dark regime at natural light intensities. Under these standard control conditions, the ambient temperature was ~ 12 °C; a thermal gradient (12 – 35 °C) was obtained through a low wattage light bulb (09:00 – 17:00) over one end of the cage. Containers were repositioned within the room several times weekly to minimise position effects. The lizards were fed with *Tenebrio* larvae (mealworms), meaty cat food or pear-banana baby food three times per week. A calcium supplement was dusted over their food at regular intervals, and multi-vitamin supplements were supplied in the drinking water, which was available *ad libitum*.

Experimental treatments

For each population, the treatments were initiated simultaneously when the first female gave birth to a healthy live litter, signifying that the remainder of the females were close to giving birth (there is only a three-four week spread in the timing of births in the natural population (Wapstra *et al.*, 1999)): this female became

the first control. The remaining females were distributed randomly into treatment groups comprising: controls (no change in standard conditions); and females held at 10 °C with no opportunity for thermoregulation, in an air-conditioned room with the same photoperiod, for one, two, or three weeks (see below). This temperature was chosen to mimic poor summer weather in Tasmania, during which little activity is possible for lizards. During their period in cold conditions, females were provided only with water *ad libitum*, as the animals do not eat at this temperature. At the completion of each group's period in the cold treatment, the females were returned to the standard control conditions in the laboratory.

Females were checked several times daily from the start of the treatment; both females and neonates from the cold treatment groups were returned immediately to the control laboratory after parturition. At parturition, maternal snout-vent length (SVL) and mass were measured. For litters of two or more neonates (litter sizes see below), one was sacrificed for determination of abdominal fat body mass (wet and dry) and body mass (wet and dry): dry masses were obtained by drying samples in an oven (~70 °C) for approximately five days, and SVL. For all remaining offspring: mass, SVL, tail and total length were measured. Sprint speed was measured, on the day of birth, using a computerised racetrack: animals warmed to 26 °C (Melville, 1998) for 30 min were run twice down a 50 cm track also heated to 26 °C, with the fastest speed being used for analysis (as a measure of “best performance”). To avoid the problem of non-independence of data collected from siblings, we used litter averages to analyse differences in offspring characteristics. If births resulted in stillborns, or if neonates died within several days of birth, their data were not used. Body condition (Student residuals from the mass/SVL regression, generated from all

offspring from that population and year) was determined for each of the treatment groups, for both the offspring and the adult females.

Experiment A: In the first year of the study season (2002/2003), 60 late-term pregnant females were collected from the Central Plateau in mid-January (litter mean \pm SE: 3.1 ± 0.2 , range: 1-5). The treatment groups ($n = 20$) used were: controls, one and three weeks at 10 °C.

Experiment B: In the second year of the study season (2003/2004), 60 late-term pregnant females were collected from the Central Plateau in mid-January (litter mean \pm SE: 2.6 ± 0.2 , range: 1-6). For this experiment we reduced the longer deferral period to two weeks only, because in Experiment A, the three week treatment females produced mainly stillborns. The treatment groups ($n = 20$) used were, consequently, controls, one and two weeks at 10 °C.

Experiment C: In the second year of the study season (2003/2004), 80 late-term pregnant females were collected from Orford in mid-December (litter mean \pm SE: 1.8 ± 0.1 , range: 1-3). The treatment groups ($n = 20$) used were: controls, one, two and three weeks at 10 °C. The results of a similar experiment in the first year of this study (2002/ 2003) were confounded by an outbreak of mites (see Atkins & Wapstra, 2004 - App. 1) and are therefore not presented here. However, that trial did indicate that females from this lowland population were able to produce viable offspring after three weeks at 10 °C: thus we included a three week deferral treatment in this experiment using this population.

Survivorship, dispersal and growth of offspring

Offspring were held for up to five days in similar conditions to the pregnant control females, and were fed on alternate days with either nematodes (white worms) or pear-banana baby food. The offspring were toe-clipped to provide a unique identity and released at twelve, equally sized, continuous marked release sites (randomly selected, with siblings released at different release sites; releases occurred at population of origin) at a well defined population (Wapstra & Swain, 2001) near where the pregnant females were collected. We returned after winter to the Central Plateau site in November in 2003, and the Orford site in September/October in 2004. During a four week period, we attempted to capture all juvenile lizards at the release field site by searching the sites randomly, and as there was no expectation of long-range dispersal (Wapstra unpublished data) only the direct periphery of the population was searched. Recaptured offspring were marked temporarily with a non-toxic paint pen to avoid further recapture. When no unmarked offspring were observed at the field site, we were confident that our recaptures represented a significant proportion of surviving offspring and that non-captures were likely to be non-survivors. Upon recapture, position was noted with a GPS (to determine dispersal distance from their known GPS release site), and offspring mass, SVL, and total length were measured (total length was not measured if a tail break was evident, and mass could not be measured in windy conditions). Growth was determined by the difference in mass or length between the measurements taken upon recapture and the measurements taken at birth. We did not assess survivorship at the Central Plateau site in the second year because the offspring of the one week treatment group represented an insufficient sample size.

Statistical analyses

Data are presented as mean \pm standard error. All analyses were performed in SAS (v 6.12). Analysis of variance (ANOVA) or analysis of covariance (ANCOVA – where the covariate (maternal or neonatal) was determined by regression analyses) was used to investigate treatment effects on offspring characteristics at birth, maternal condition, dispersal distances and growth. Significant differences between the treatment groups and the control group (*a priori* comparisons) were identified using the CONTRAST statement. As a result of the large number of tests conducted, the significance level used (0.05) was adjusted to 0.005 (0.05/10 tests) (Bonferroni correction (Rao, 1998)) when testing the significance of treatment effect on offspring and maternal characteristics measured at birth. Homogeneity of group variances was assessed by studying a plot of standard deviations against means, while assumptions of normality were checked by examining plots of standardised residuals against estimated values and the normal probability curve of the residuals. Where necessary, data were log-transformed to stabilise variances and/or normalise data. Chi-squared analysis was used to analyse for differences in outcomes of pregnancy from the treatment groups (see Results), and for treatment effects on over-winter survival of the offspring; Fisher's Exact test was also used to assess over-winter survival.

RESULTS

Placing pregnant females into cold conditions near the end of gestation resulted in a delay in the timing of parturition relative to control females for both high and low elevation populations (Figure 1). For all experiments, there was no

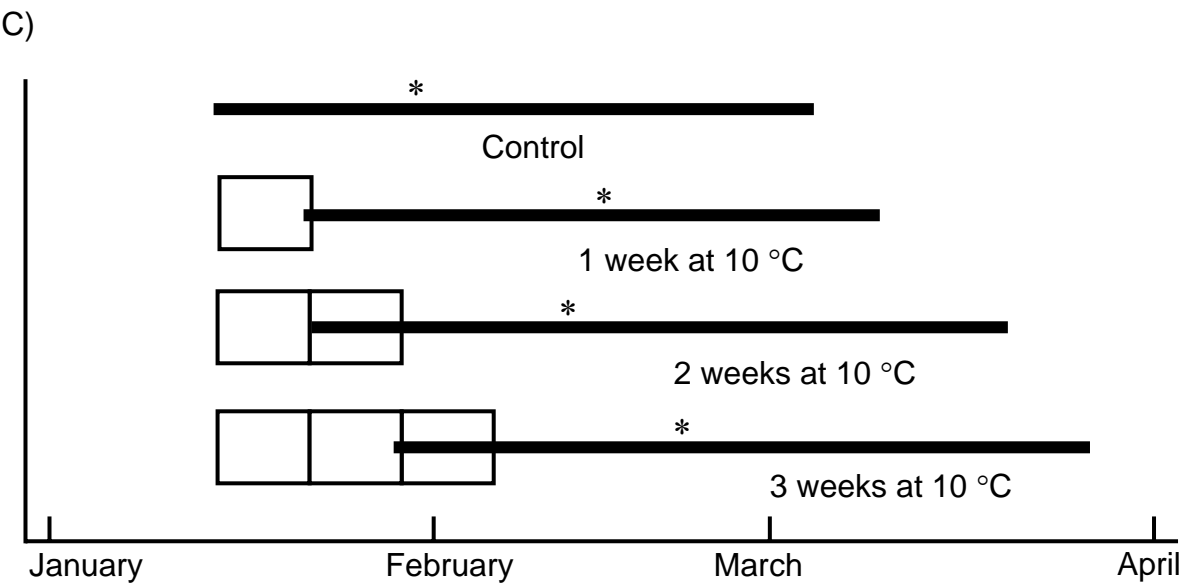
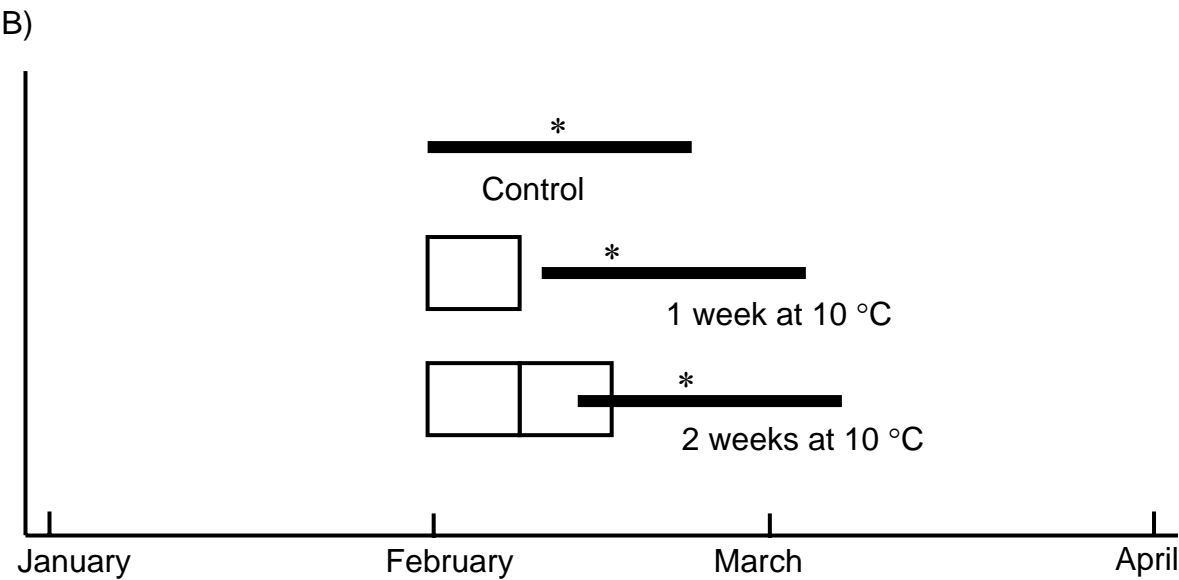
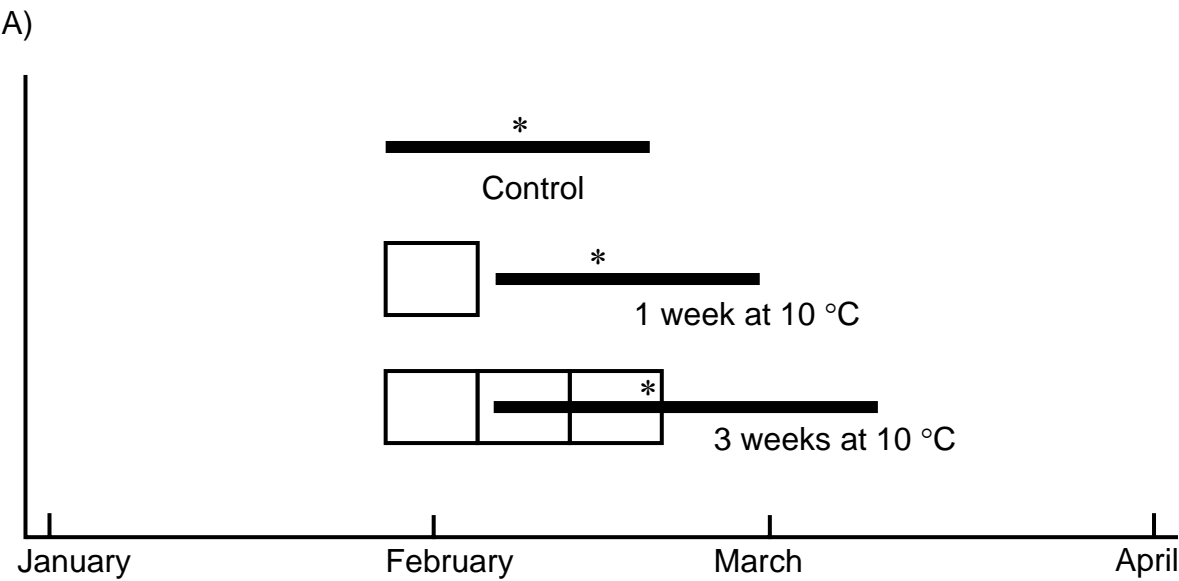


Figure 1 – Treatment effect on the timing of births in each of the populations. A) Experiment A – high elevation population (2002/2003), B) Experiment B – high elevation population (2003/2004), C) Experiment C – low elevation population (2003/2004). Solid lines represent time between first and last litter for each respective treatment group. Squares represent one week at 10 °C. * represents mean birth date for each group.

effect of treatment on maternal condition measured at the time of parturition ($P > 0.005$; Bonferroni corrected P value) (Table 1). Across all experiments, with only a single exception (i.e. low elevation population), the experimental females did not begin parturition until the second week of the experiment (Figure 1), while a third to a half of the control females did give birth within the first week.

Sample sizes for offspring data sets were reduced in all treatment groups due to differing outcomes of pregnancy. Pregnancy ended in one of five ways: 1) fully developed viable neonates at stage 40 (Dufaure & Hubert, 1961); 2) litters of apparently viable neonates that only survived one or two days after birth; 3) litters of stillborn neonates or neonates that died within several hours of birth (stage 40); 4) abortions of partially developed neonates ($< \text{stage } 40$), and; 5) cases where no product of pregnancy was observed. In the latter case the female probably consumed the neonates before parturition could be detected (Atkins & Jones, unpublished observations, see also Lourdais *et al.*, 2005). Sample sizes were especially reduced in Experiment B (high elevation population during 2003/2004) because 13 litters of either stillbirths or the presence of after-birth only were observed before the birth of the first litter of viable neonates (and therefore the start of the experiment).

Table 1 - Offspring and maternal characteristics measured at birth. A) Experiment A - high elevation population (2002/2003), B) Experiment B - high elevation population (2003/2004), C) Experiment C - low elevation population (2003/2004). Values are means for each litter \pm SE (n).

A)	Control	1 week at 10 °C	2 weeks at 10 °C	3 weeks at 10 °C
Neonate mass - wet (mg)	554.15 \pm 17.36 (13)	563.44 \pm 12.47 (14)	~	531.43 \pm 20.08 (7)
Neonate mass - dry (mg)	97.12 \pm 3.35 (13)	102.02 \pm 3.27 (13)	~	98.18 \pm 5.13 (5)
Neonate SVL (mm)	29.04 \pm 0.20 (13)	29.35 \pm 0.21 (14)	~	28.71 \pm 0.36 (7)
Neonate condition (residuals)	0.09 \pm 0.29 (13)	-0.05 \pm 0.26 (14)	~	-0.07 \pm 0.46 (7)
Neonate tail length (mm)	36.82 \pm 0.61 (13)	37.14 \pm 0.35 (14)	~	35.59 \pm 0.53 (7)
Neonate total length (mm)	65.98 \pm 0.81 (13)	66.74 \pm 0.50 (14)	~	64.71 \pm 0.71 (7)
Neonate fat bodies - wet (mg)	9.34 \pm 1.17 (13)	7.83 \pm 0.50 (13)	~	7.84 \pm 1.75 (5)
Neonate fat bodies - dry (mg)	4.69 \pm 0.80 (13)	4.50 \pm 0.56 (13)	~	5.62 \pm 1.59 (5)
Neonate sprint speed (cm/s)	32.73 \pm 1.98 (13)	30.28 \pm 1.50 (14)	~	32.27 \pm 4.65 (7)
Maternal condition (residuals)	0.12 \pm 0.26 (19)	-0.16 \pm 0.27 (16)	~	0.02 \pm 0.20 (18)
B)				
Neonate mass - wet (mg)	546.48 \pm 11.83 (13)	552.99 \pm 31.73 (6)	528.88 \pm 14.83 (11)	~
Neonate mass - dry (mg)	91.16 \pm 2.24 (12)	89.46 \pm 9.07 (5)	92.33 \pm 4.23 (10)	~
Neonate SVL (mm)	29.25 \pm 0.20 (13)	29.17 \pm 0.49 (6)	29.26 \pm 0.21 (11)	~
Neonate condition (residuals)	0.17 \pm 0.20 (13)	0.59 \pm 0.32 (6)	-0.53 \pm 0.37 (11)	~
Neonate tail length (mm)	36.62 \pm 0.37 (13)	37.40 \pm 0.88 (6)	35.91 \pm 0.45 (11)	~
Neonate total length (mm)	65.87 \pm 0.49 (13)	66.68 \pm 1.33 (6)	65.22 \pm 0.61 (11)	~
Neonate fat bodies - wet (mg)	6.53 \pm 0.51 (12)	7.08 \pm 1.43 (5)	5.89 \pm 1.16 (10)	~
Neonate fat bodies - dry (mg)	3.97 \pm 0.63 (12)	4.22 \pm 1.04 (5)	3.64 \pm 0.73 (10)	~
Neonate sprint speed (cm/s)	31.68 \pm 2.02 (13)	30.28 \pm 3.31 (6)	27.14 \pm 1.87 (11)	~
Maternal condition (residuals)	0.04 \pm 0.22 (15)	0.30 \pm 0.35 (11)	-0.28 \pm 0.27 (14)	~
C)				
Neonate mass - wet (mg)	484.54 \pm 17.27 (16)	438.80 \pm 18.48 (15)	460.93 \pm 18.28 (12)	437.06 \pm 16.51 (15)
Neonate mass - dry (mg)	83.95 \pm 3.08 (12)	70.65 \pm 3.57 (10)	79.65 \pm 3.09 (11)	72.93 \pm 3.46 (10)
Neonate SVL (mm)	28.24 \pm 0.36 (16)	27.79 \pm 0.30 (15)	27.55 \pm 0.32 (12)	27.50 \pm 0.25 (15)
Neonate condition (residuals)	0.20 \pm 0.25 (16)	-0.45 \pm 0.24 (15)	0.46 \pm 0.32 (12)	-0.12 \pm 0.23 (15)
Neonate tail length (mm)	36.21 \pm 0.63 (16)	35.15 \pm 0.61 (13)	35.66 \pm 0.71 (12)	33.66 \pm 0.75 (15)
Neonate total length (mm)	64.53 \pm 0.96 (16)	63.00 \pm 0.84 (13)	63.81 \pm 0.96 (12)	61.22 \pm 0.97 (15)
Neonate fat bodies - wet (mg)	4.83 \pm 0.55 (12)	3.06 \pm 0.63 (9)	3.47 \pm 0.60 (11)	3.04 \pm 0.56 (10)
Neonate fat bodies - dry (mg)	3.22 \pm 0.44 (12)	1.56 \pm 0.52 (10)	2.17 \pm 0.47 (11)	1.63 \pm 0.42 (10)
Neonate sprint speed (cm/s)	30.66 \pm 2.06 (16)	27.31 \pm 2.19 (14)	30.87 \pm 2.98 (12)	27.42 \pm 1.80 (15)
Maternal condition (residuals)	-0.15 \pm 0.22 (18)	0.32 \pm 0.24 (17)	0.25 \pm 0.25 (18)	0.38 \pm 0.21 (19)

Experiment A – outcomes of parturition

The length of time between the first litter and the last litter within each treatment group was similar (22 days) for the controls and the one week females, but this interval was extended to 32 days in the three week treatment group (Figure 1A). In these females from the high elevation population (season 2002/2003), all births in the one week treatment group occurred after return to standard laboratory conditions as mentioned above (Figure 1A, Table 2A). The group experiencing three weeks in cold conditions reacted differently: the majority of births (13 of 18) occurred while still under cold conditions, and approximately half of these births produced unviable offspring. Significantly more unviable litters occurred in the three week treatment group ($\chi^2 = 6.637$, $P = 0.036$). The stillborn offspring (including the neonates that only survived one to two hours after birth) from the three week treatment group were smaller than the viable neonates from the same experiment (Table 1A) (stillbirth litter means: dry mass of 81.1 ± 5.01 mg (10), SVL of 25.63 ± 0.57 mm (9)). The mean dry weight of the fat bodies (4.69 ± 0.64 (9)) of these stillborns was similar to that of the controls (4.69 ± 0.8 (13)) and was not significantly different from that of the viable neonates from the same treatment group ($P > 0.05$).

Experiment B – outcomes of parturition

The interval of time from the first litter to the last litter within each experimental group was similar for all three groups (20, 20 and 21 days respectively) (Figure 1B). In these females from the high elevation population (season 2003/2004), subjected to one week at 10 °C, all births occurred after the return to control laboratory conditions (Figure 1B, Table 2B). In the females subjected to two weeks under cold conditions, three births occurred during the cold period of the treatment,

Table 2 - The spread of births over the experiment. A) Experiment A - high elevation population (2002/2003), B) Experiment B - high elevation population (2003/2004), C) Experiment C - low elevation population (2003/2004).

A)

Treatment	No. births in cold conditions			No. births in standard conditions		
	Viable	Non-viable	Total	Viable	Non-viable	Total
Control	N/A	N/A		15	4	19
1 week at 10 °C	0	0	0	14	2	16
3 weeks at 10 °C	6	7	13	3	2	5

B)

Control	N/A	N/A		13	2	15
1 week at 10 °C	0	0	0	6	7	13
2 weeks at 10 °C	2	1	3	9	3	12

C)

Control	N/A	N/A		16	3	19
1 week at 10 °C	1	0	1	14	3	17
2 weeks at 10 °C	4	0	4	10	4	14
3 weeks at 10 °C	4	1	5	11	3	14

but the majority of births (12 of 15) occurred after the return to control laboratory conditions. There was no effect of treatment on viability of litters ($P > 0.05$).

Experiment C – outcomes of parturition

The length of time from the first litter to the last litter within each of the experimental groups was similar in the control and one week group (48 and 45 days respectively) (Figure 1C). However, the range of birth dates increased markedly, to 55 days in the two week treatment group, and 56 days in the three week treatment group. In these females from the low elevation population (season 2003/2004) subjected to cold conditions, the majority of births occurred after removal from the cold conditions (Table 2C). For this experiment, the births (one exception) that did occur while under cold conditions resulted in viable neonates. For the two and three week treatment groups, the first two and three females respectively that gave birth after removal from the cold conditions produced unviable litters, with all subsequent litters viable. However, there was no effect of treatment on viability of litters ($P > 0.05$).

Offspring characteristics at birth

In all experiments, for all the offspring characteristics measured (Table 1), there was no significant effect of treatment on mass at birth (wet and dry), fat body mass (wet and dry), SVL, tail length, total length, condition or sprint speed ($P > 0.005$; Bonferroni corrected P value).

Survival of offspring over winter

The effect of treatment on offspring survival (Table 3) was assessed by determining the number of females in each treatment group represented by surviving offspring recaptured after winter (either one or two offspring), and comparing this with the total number of females in that treatment group which were represented by released offspring. There was no significant effect of treatment on survivorship in experiment A ($\chi^2 = 1.488$, $P = 0.475$; Fisher's Exact test, $P = 0.569$) (Table 3A). Assessing survivorship in the low elevation population (experiment C) was hampered by low recapture rates and lower litter sizes; there were no significant effects of treatment on survivorship in Experiment C ($\chi^2 = 0.857$, $P = 0.836$; Fisher's Exact test, $P = 0.863$) (Table 3B).

Dispersal and growth of offspring after release

Offspring from the high elevation population dispersed 1.4 – 54.0 m from their release sites (16.5 ± 2.54 m, $N = 24$), and offspring from the low elevation population dispersed 4.0 – 75.3 m from their release sites (19.9 ± 4.55 m, $N = 17$) (Table 4).

Due to a high incidence of females in the high elevation population being represented by more than one surviving offspring, and lower sample sizes in the low elevation population (both a consequence of litter size differences between the populations), we conducted dispersal and growth analyses on individual offspring rather than litter means: this enabled us to keep sample sizes as high as possible within each of the treatment groups (as in Olsson & Shine, 2003). Dispersal distances were very variable, and there was no effect of treatment on dispersal distances in either of the populations ($P > 0.05$). As there was no significant effect when

Table 3 - Survivorship of offspring over winter. A) Experiment A - high elevation population (2002/2003), B) Experiment C - low elevation population (2003/2004)

A)

	Control	1 week at 10 °C	2 weeks at 10 °C	3 weeks at 10 °C
Number of females with surviving offspring (1 or 2)	10 (71%)	7 (50%)	~	2 (25%)
Number of females that gave birth	14	14	~	8

B)

Number of females with surviving offspring (1 or 2)	6 (38%)	4 (25%)	3 (21%)	3 (20%)
Number of females that gave birth	16	16	14	15

Table 4 - Dispersal distance and increase in length or mass over winter. A) Experiment A - high elevation population (2002/2003), B) Experiment C - low elevation population (2003/2004). Values are means for each litter \pm SE (*n*). Due to insufficient data, results were unable to be generated for total length and mass in Experiment A for the 3 week treatment group, and for total length in Experiment C for both the 2 and 3 week treatment groups.

A)

	Control	1 week at 10 °C	2 weeks at 10 °C	3 weeks at 10 °C
Distance (m)	17.84 \pm 2.73 (12)	17.79 \pm 5.50 (9)	~	6.02 \pm 1.83 (3)
Snout-vent length (mm)	8.63 \pm 0.67 (12)	7.79 \pm 0.70 (9)	~	7.80 \pm 1.78 (3)
Total length (mm)	20.31 \pm 1.97 (10)	17.16 \pm 2.68 (6)	~	
Mass (mg)	340.13 \pm 56.98 (8)	192.50 \pm 47.92 (6)	~	

B)

Distance (m)	27.91 \pm 8.70 (7)	19.10 \pm 8.99 (4)	21.39 \pm 9.54 (3)	6.24 \pm 1.37 (3)
Snout-vent length (mm)	10.72 \pm 1.43 (7)	9.48 \pm 0.93 (4)	6.11 \pm 0.45 (3)	4.79 \pm 0.90 (3)
Total length (mm)	34.09 \pm 3.09 (5)	29.29 \pm 6.73 (4)		

analysing offspring as if they were statistically independent, we did not take the further step of analysing these data more stringently (i.e. taking into account non-independence of siblings by considering one selected offspring per family and performing resampling as in Massot *et al.*(1994)).

There was no significant treatment effect on growth, either SVL or total length measured after winter in spring, in the high elevation population ($P > 0.05$). There was, however, a significant treatment effect on mass increase measured after winter in spring in this population (ANCOVA, $F_{(1,11)} = 5.81$, $P = 0.0346$, day of recapture as covariate), with offspring in the one week treatment group increasing in mass less than offspring from the control group. As a consequence of insufficient sample sizes in the three week treatment group, total length and mass changes could not be assessed. In the low elevation population, there was a significant treatment effect on growth (increase in SVL) measured after winter in spring (ANCOVA, $F_{(3,12)} = 4.21$, $P = 0.0299$, log transformed, day of recapture as covariate), with significantly lower growth of the offspring of both the two and three week treatment groups when compared to the controls ($P < 0.05$). There was no effect of treatment on the increase in total length of the offspring ($P > 0.05$): this factor could only be compared between the control and one week treatment groups due to insufficient sample sizes in the other treatment groups. For similar reasons, analyses of changes in offspring mass are not presented.

DISCUSSION

This study has demonstrated, first, that females of the viviparous lizard *Niveocincus ocellatus* are able to defer parturition if subjected to adverse (cold)

environmental conditions near the end of gestation. Second, our results demonstrate that there are no subsequent effects on either maternal condition or offspring phenotypic characters measured at birth, nor on their dispersal or survivorship. The ability to defer parturition successfully after completion of embryonic development may therefore represent an important strategy for maximising offspring fitness in viviparous lizards. The limited published work to date shows that in *N. metallicus* parturition date can be delayed experimentally by at least three weeks with no decrease in the viability of the neonates (Swain & Jones, 2000b), and both *N. microlepidotus* and the gecko *Hoplodactylus maculatus* defer birth naturally for many months over winter (Cree & Guillelte, 1995; Hutchinson *et al.*, 1989; Olsson & Shine, 1998). These observations contrast with those of mammals, in which gestation can be delayed by only a few hours to a few days under experimental conditions in which hormones are administered (Aiken, 1972; Challis, Davies & Ryan, 1975; Chester *et al.*, 1972).

However, in our species, *N. ocellatus*, the ability to defer parturition differed between the populations from high and low elevations, representing each extreme of the species' distribution. Our hypothesis, that females of the high elevation population of *N. ocellatus* will be less likely to defer birth under extended periods of poor conditions, was supported by our results. In this population, the constraints of the shorter breeding season reduce the advantages of being able to defer birth in response to proximate environmental variation. Similarly, bird species living at high latitudes are less sensitive to environmental perturbations during the breeding season than comparable species at lower latitudes (Jacobs & Wingfield, 2000); the birds experiencing a shorter breeding season in a severe environment rely on one reliable environmental cue to time reproduction.

However, what are the implications of deferred birth for offspring fitness in our species? In general, females from the low elevation population are able both to successfully defer birth under extended periods (three weeks) of adverse conditions, and to produce viable offspring. This supports our hypothesis that deferral of parturition represents an important strategy for optimising offspring fitness in viviparous lizards, particularly those which may experience uncertain environmental conditions towards the end of gestation. In contrast, exposing females from the high elevation population to longer periods of cold did affect offspring viability. Most births occurred while the females were in the cold conditions, and most of these offspring were not viable. This suggests that in the high elevation population there has been limited selection for the ability to defer parturition.

For females of the high elevation population, the stillborns produced by the three week treatment group were smaller than the viable neonates, but had similarly sized fat bodies, suggesting that there was little difference in “condition”. Rock and Cree (2003) observed a similar phenomenon in the gecko *H. maculatus* when comparing viable and stillborn neonates produced under cool experimental conditions. Our results thus, suggest that the embryos (or early neonates) did not die because their energy reserves were depleted. Many aspects of reptilian embryonic development are sensitive to temperature (Beuchat, 1986), and the length and success of gestation can be affected by maternal temperature during gestation (Beuchat, 1986; Swain & Jones, 2000a; Wapstra, 2000). In our experiment, females were placed into their treatment groups immediately after the first birth in the laboratory, but some variation in the degree of completion of embryonic development must be expected. The period of time over which parturition occurs (and therefore the “spread” of embryonic development) is shorter at the Central Plateau (Wapstra *et al.*,

1999), and this could, therefore, expose a greater percentage of embryos, relative to the low elevation population, at a particularly sensitive stage to the adverse conditions experienced by the female. In *S. jarrovi*, Guillette, DeMarco & Palmer (1991) experimentally prolonged pregnancy by treatment with either progesterone or indomethacin, and reported a high incidence of dead but fully formed *in utero* young. They speculated that the extended retention of young might account for the large number of *in utero* deaths, and suggested that these deaths were due primarily to asphyxia. We therefore propose that, in our study, the higher occurrence of stillbirths in the high elevation population in the three week treatment may reflect an inability of the females from this population to hold fully developed embryos for longer than two weeks without loss of viability.

However, in both populations of *N. ocellatus*, placing females in cold conditions (10 °C, with no opportunity to thermoregulate) for up to 3 weeks near the end of gestation had no significant effect on the offspring characteristics (of the viable offspring only) that we measured. This is perhaps not surprising, as treatments were started when embryonic development was nearly complete, and in this ectothermic species metabolism and growth would have been depressed by the low temperatures. In a similarly run experiment (Swain & Jones, 2000a) on *N. metallicus*, there were also no significant differences in neonate mass, SVL and sprint speed at birth between treatment groups. In *N. ocellatus*, delaying birth by up to three weeks again did not significantly affect either body condition or energy reserves (abdominal fat bodies) of the neonates; thus, on these criteria, neonates are not disadvantaged by their mother delaying their birth. Furthermore, offspring are born into a more favourable environment, with an improved chance of survival and ability to disperse.

For both of the populations, however, there was a treatment effect on growth of the offspring after release. These treatment effects on growth, if they persist, could have future consequences for size and age at maturity, and, hence, fecundity (Wapstra *et al.*, 2001). With no significant differences in offspring phenotype at birth, the significant treatment effects on growth in both populations are, presumably, a consequence of the shifted birth date (and therefore release date), which has been shown to influence offspring growth rate (Olsson & Shine, 1997; Sinervo & Doughty, 1996). This result illustrates the importance of, both, assessing offspring after birth and performing a field-based assessment of treatment consequences for offspring fitness.

In conclusion, females of the viviparous skink *N. ocellatus* subjected to adverse environmental conditions during the latter stages of gestation are able to defer parturition to some degree, but this varies between populations. Our hypothesis that females from the high elevation population would be less able to defer parturition (thus ensuring that offspring were not born too late in the season) was supported. For all viable offspring born, there was no treatment effect on offspring phenotype at birth, nor dispersal distance or survivorship after release. However, a treatment effect on growth after release was detected in both populations, suggesting that there are trade-offs between the benefits of deferral of parturition and birth date. Implicit in these arguments is the assumption that birth during poor environmental conditions is detrimental. This has not been specifically tested in this, nor, to our knowledge, in any other system; in future this could be tested by comparing offspring born and released into poor conditions with and without deferral of parturition.

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REFERENCES

- Aiken JW. 1972.** Aspirin and indomethacin prolong parturition in rats: evidence that prostaglandins contribute to expulsion of foetus. *Nature* 240: 21-25.
- Andrews RM, Rose BR. 1994.** Evolution of viviparity: constraints on egg retention. *Physiological Zoology* 67: 1006-1024.
- Atkins N, Wapstra E. 2004.** Successful treatment of a mite infestation in gravid spotted snow skinks (*Niveoscincus ocellatus*). *Herpetofauna* 34: 66-69.
- Bernado J. 1991.** Manipulating egg size to study maternal effects on offspring traits. *Trends in Ecology and Evolution* 6: 1-2.
- Bernado J. 1996.** Maternal effects in animal ecology. *American Zoologist* 36: 83-105.
- Beuchat CA. 1986.** Reproductive influences on the thermoregulatory behaviour of a live-bearing lizard. *Copeia*. 1986: 971-979.
- Bowen WD, Ellis SL, Iverson SJ, Boness DJ. 2003.** Maternal and newborn life-history traits during periods of contrasting population trends: implications for explaining the decline of harbour seals (*Phoca vitulina*), on Sable Island. *Journal of Zoology (London)* 261: 155-163.

- Challis JRG, Davies IJ, Ryan KJ. 1975.** The effects of dexamethasone and indomethacin on the outcome of pregnancy in the rabbit. *Journal of Endocrinology* 64: 363-370.
- Chester R, Dukes M, Slater SR, Walpole AI. 1972.** Delay of parturition in the rat by anti-inflammatory agents which inhibit the biosynthesis of prostaglandins. *Nature* 240: 37-38.
- Civantos E, Salvador A, Veiga JP. 1999.** Body size and microhabitat affect winter survival of hatchling *Psammodromus algirus* lizards. *Copeia* 1999: 1112-1117.
- Clobert J, Massot M, Lecomte J, Sorci G, de Fraipont M, Barbault R. 1994.** Determinants of dispersal behaviour: The common lizard as a case study. In: Vitt LJ and Pianka ER, eds. *Lizard Ecology: Historical and Experimental Perspectives*. Princeton, New Jersey: Princeton University Press. 183-206.
- Cree A, Guillelte JL Jr. 1995.** Biennial reproduction with a fourteen-month pregnancy in the gecko *Hoplodactylus maculatus* from southern New Zealand. *Journal of Herpetology* 29: 163-173.
- Doughty P, Sinervo B. 1994.** The effects of habitat, time of hatching, and body size on the dispersal of hatchling *Uta stansburiana*. *Journal of Herpetology* 28: 485-490.
- Doughty P, Sinervo B, Burghardt GM. 1994.** Sex-biased dispersal in a polygynous lizard, *Uta stansburiana*. *Animal Behaviour* 47: 227-229.
- Du WG, Shou L, Liu JK. 2003.** The effect of incubation temperature on egg survival, hatchling traits and embryonic use of energy in the blue-tailed skink, *Eumeces elegans*. *Animal Biology* 53: 27-36.

- Dufaure JP, Hubert J. 1961.** Table de development du lezard vivipare: *Lacerta* (*Zootoca*) *vivipara* Jaquin. *Archives d'Anatomie Microscopique et de Morphologie Experimentale* 50: 309-328.
- Elphick MJ, Shine R. 1998.** Longterm effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards (*Bassiana duperreyi*, Scincidae). *Biological Journal of the Linnean Society* 63: 429-447.
- Girling JE, Jones SM, Swain R. 2002.** Delayed ovulation and parturition in a viviparous alpine lizard (*Niveoscincus microlepidotus*): morphological data and plasma steroid concentrations. *Reproduction Fertility and Development* 14: 43-53.
- Guillette JL Jr, DeMarco V, Palmer BD. 1991.** Exogenous progesterone or indomethacin delays parturition in the viviparous lizard *Sceloporus jarrovi*. *General and Comparative Endocrinology* 81: 105-112.
- Hare KM, Daugherty CH. 2002.** Incubation regime affects juvenile morphology and hatching success, but not sex, of the oviparous lizard *Oligosoma suteri* (Lacertilia: Scincidae). *New Zealand Journal of Zoology* 29: 221-229.
- Hutchinson MN, Robertson P, Rawlinson PA. 1989.** Redescription and ecology of the two endemic Tasmanian scincid lizards *Leiopisma microlepidotum* and *L. pretiosum*. *Papers and Proceedings of the Royal Society of Tasmania* 123: 257-274.
- Jacobs JD, Wingfield J. 2000.** Endocrine control of life-cycle stages: a constraint on response to the environment? *The Condor* 102: 35-51.
- Licht P. 1984.** Reptiles. In: Lamming GE, ed. *Marshall's Physiology of Reproduction*. 4th ed. Edinburgh, London, New York, Melbourne: Churchill Livingston. 206-282.

-
- Lloyd JD, Martin TE. 2004.** Nest-site preference and maternal effects on offspring growth. *Behavioral Ecology* 15: 816-823.
- Lourdais O, Brischoux F, Shine R, Bonnet X. 2005.** Adaptive maternal cannibalism in snakes (*Epicrates cenchria maurus*, Boidae). *Biological Journal of the Linnean Society* 84: 767-774.
- Massot M, Clobert J. 1995.** Influence of maternal food availability on offspring dispersal. *Behavioral Ecology & Sociobiology* 37: 413-418.
- Massot M, Clobert J. 2000.** Processes at the origin of similarities in dispersal behaviour among siblings. *Journal of Evolutionary Biology* 13: 707-719.
- Massot M, Clobert J, Chambon A, Michalakis Y. 1994.** Vertebrate natal dispersal: the problem of non-independence of siblings. *Oikos* 70: 172-176.
- Mathies T, Andrews RM. 1995.** Thermal and reproductive biology of high and low elevation populations of the lizard *Sceloporus scalaris* - implications for the evolution of viviparity. *Oecologia* 104: 101-111.
- Melville J. 1998.** The evolution of locomotory mode in the lizard genus *Niveoscincus*: An ecomorphological analysis of ecology, behaviour, morphology and performance ability. Unpublished D. Phil. Thesis, University of Tasmania.
- Mousseau TA, Fox CW. 1998.** The adaptive significance of maternal effects. *Trends in Ecology & Evolution* 13: 403-407.
- Olsson M, Gullberg A, Tegelstrom H. 1996.** Malformed offspring, sibling matings, and selection against inbreeding in the sand lizard (*Lacerta agilis*). *Journal of Evolutionary Biology* 9: 229-242.

-
- Olsson M, Shine R. 1996.** Does reproductive success increase with age or with size in species with indeterminate growth? A case study using sand lizards (*Lacerta agilis*). *Oecologia* 105: 175-178.
- Olsson M, Shine R. 1997.** The seasonal timing of oviposition in sand lizards (*Lacerta agilis*): why early clutches are better. *Journal of Experimental Zoology* 10: 369-381.
- Olsson M, Shine R. 1998.** Timing of parturition as a maternal care tactic in an alpine lizard species. *Evolution* 52: 1861-1864.
- Olsson M, Shine R. 1999.** Plasticity of frequency of reproduction in an alpine lizard, *Niveoscincus microlepidotus*. *Copeia* 1999: 794-796.
- Olsson M, Shine R. 2003.** Female-based natal and breeding dispersal in an alpine lizard, *Niveoscincus microlepidotus*. *Biological Journal of the Linnean Society* 79: 277-283.
- Poussart C, Gauthier G, Larochelle J. 2001.** Incubation behaviour of greater snow geese in relation to weather conditions. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 79: 671-678.
- Qvarnstrom A, Price TD. 2001.** Maternal effects, paternal effects and sexual selection. *Trends in Ecology & Evolution* 16: 95-100.
- Radder RS, Shanbhag B, Saidapuir SK. 1998.** Prolonged oviductal egg retention arrests embryonic growth at stage 34 in captive *Calotes versicolor*. *Herpetological Review* 29: 217-218.
- Rao PV. 1998.** *Statistical research methods in the life sciences*. Brooks/Cole Publishing Company, Pacific Grove, USA.

-
- Rawlinson PA. 1974.** Biogeography and ecology of the reptiles of Tasmania and the Bass Strait area. In: Williams WD, ed. *Biogeography and Ecology in Tasmania*. The Hague: Junk, W. 291-388.
- Reale D, McAdam AG, Boutin S, Berteaux D. 2003.** Genetic and plastic responses of a northern mammal to climate change. *Proceedings of the Royal Society of London Series B-Biological Sciences* 270: 591-596.
- Reinhold K. 2002.** Maternal effects and the evolution of behavioral and morphological characters: A literature review indicates the importance of extended maternal care. *Journal of Heredity* 93: 400-405.
- Rock J, Cree A. 2003.** Intraspecific variation in the effect of temperature on pregnancy in the viviparous gecko *Hoplodactylus maculatus*. *Herpetologica* 59: 8-22.
- Schwarzkopf L, Shine R. 1991.** Thermal biology of reproduction in viviparous skinks, *Eulamprus tympanum* - why do gravid females bask more. *Oecologia* 88: 562-569.
- Shanbhag BA, Saidapur SK, Radder RS. 2003.** Lowering body temperature induces embryonic diapause during prolonged egg retention in the lizard, *Calotes versicolor*. *Naturwissenschaften* 90: 33-35.
- Shine R. 1995.** A new hypothesis for the evolution of viviparity in reptiles. *The American Naturalist* 145: 809-823.
- Shine R, Downes SJ. 1999.** Can pregnant lizards adjust their offspring phenotypes to environmental conditions? *Oecologia* 119: 1-8.
- Shine R, Elphick MJ, Harlow PS. 1997.** The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology* 78: 2559-2568.

-
- Shine R, Harlow P. 1993.** Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia* 96: 122-127.
- Shine R, Harlow PS. 1996.** Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology* 77: 1808-1817.
- Shine R, Olsson M. 2003.** When to be born? Prolonged pregnancy or incubation enhances locomotor performance in neonatal lizards (Scincidae). *Journal of Evolutionary Biology* 16: 823-832.
- Sinervo B, Doughty P. 1996.** Interactive effects of offspring size and timing of reproduction on offspring reproduction: experimental, maternal, and quantitative genetic aspects. *Evolution* 50: 1314-1327.
- Sorci G, Clobert J. 1997.** Environmental maternal effects on locomotor performance in the common lizard (*Lacerta vivipara*). *Evolutionary Ecology* 11: 531-541.
- Stewart JR. 1989.** Facultative placentotrophy and the evolution of squamate placentation: quality of eggs and neonates in *Virginia striatula*. *The American Naturalist* 133: 111-137.
- Swain R, Jones SM. 2000a.** Facultative placentotrophy: half-way house or strategic solution? *Comparative Biochemistry and Physiology Part A* 127: 441-451.
- Swain R, Jones SM. 2000b.** Maternal effects associated with gestation conditions in a viviparous lizard, *Niveoscincus metallicus*. *Herpetological Monographs* 14: 432-440.
- Tarboton WR. 1993.** Incubation behavior of the African jacana. *South African Journal of Zoology* 28: 32-39.
- Thompson MB, Speake BK, Stewart JR, Russell K, McCartney RJ, Surai PF. 1999a.** Placental nutrition in the viviparous lizard *Niveoscincus metallicus*:

the influence of placental type. *Journal of Experimental Biology* 202: 2985-2997.

Thompson MB, Stewart JR, Speake BK, Russell K, McCartney RJ, Surai PF.

1999b. Placental nutrition in a viviparous lizard (*Pseudemoia pagenstecheri*) with a complex placenta. *Journal of Zoology, (London)* 248: 295-305.

Thompson MB, Stewart JR, Speake BK, Russell KJ, McCartney RJ. 1999c.

Placental transfer of nutrients during gestation in the viviparous lizard, *Pseudemoia spenceri*. *Journal of Comparative Physiology B* 169: 319-328.

Van Damme R, Bauwens D, Brana F, Verheyen RF. 1992. Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. *Herpetologica* 48: 220-228.

Vial JL, Stewart JR. 1985. The reproductive cycle of *Barisia monticola*: a unique variation among viviparous lizards. *Herpetologica* 41: 51-57.

Wapstra E. 2000. Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Functional Ecology* 14: 345-353.

Wapstra E, O'Reilly J. 2001. Potential 'costs of reproduction' in a skink: inter- and intrapopulational variation. *Austral Ecology* 26: 179-186.

Wapstra E, Swain R. 1996. Feeding ecology of the Tasmanian spotted skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Australian Journal of Zoology* 44: 205-213.

Wapstra E, Swain R. 2001. Geographic and annual variation in life-history traits in a temperate zone Australian skink. *Journal of Herpetology* 35: 194-203.

Wapstra E, Swain R, Jones SM, O'Reilly J. 1999. Geographic and annual variation in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Australian Journal of Zoology* 47: 539-550.

-
- Wapstra E, Swain R, O'Reilly J. 2001.** Geographic variation in age and size at maturity in a small Australian viviparous skink. *Copeia* 2001: 646-655.
- Warner DA, Andrews RM. 2003.** Consequences of extended egg retention in the Eastern Fence Lizard (*Sceloporus undulatus*). *Journal of Herpetology* 37: 309-314.

Part 2.

*Endocrine mechanisms controlling
parturition and their modulation by
environmental influences or beta-
adrenergic stimulation.*

Chapter 5

Timing of parturition in two species of viviparous lizard: influences of β -adrenergic stimulation and temperature upon uterine responses to arginine vasotocin (AVT)

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Abstract

The southern snow skink *Niveoscincus microlepidotus* is a viviparous alpine lizard with biennial reproduction, in which embryos are fully developed before winter but parturition is delayed until spring. We aimed to determine whether, in this species, *in vitro* uterine preparations are responsive to arginine vasotocin (AVT) and prostaglandin ($\text{PGF}_{2\alpha}$) in autumn and spring, and whether pre-treatment with the β -adrenergic agonist isoproterenol decreases the effectiveness of AVT in stimulating uterine contractions. Using the spotted snow skink (*Nivescincus ocellatus*), an annually breeding species, we aimed to determine influences of temperature and the β -adrenergic system upon the response to AVT *in vivo*. In both *N. microlepidotus* and *N. ocellatus* females are more responsive to AVT than to $\text{PGF}_{2\alpha}$, and that the response to AVT is decreased, but not prevented, by β -adrenergic stimulation. In *N. microlepidotus*, uteri are equally responsive in both seasons to the hormones

administered. In *N. ocellatus* environmental conditions, specifically, temperature, modulate the response to AVT *in vivo* with the time to parturition increasing as temperature decreases. We conclude that in these viviparous squamates the endocrine cascade leading to parturition is modulated by the β -adrenergic system, and that this may reflect the mechanism by which the timing of parturition is tied to suitable environmental conditions.

Introduction

The control of parturition (and oviposition) in reptiles involves a series of complex mechanisms that interact with each other and may also be influenced by the environment. In viviparous squamates (lizards, snakes and amphisbaenians), parturition can be delayed for weeks (e.g. *Niveoscincus metallicus*: Swain and Jones 2000) or even months (e.g. *Niveoscincus microlepidotus*: Olsson and Shine 1998, 1999) after the completion of embryonic development, while in oviparous reptiles, females will retain their eggs if environmental conditions are not suitable for oviposition (Guillette et al. 1980; Andrews and Rose 1994; Shanbhag et al. 2003). Such observations suggest that in reptiles there may be some environmental modulation either of the endocrine cascade initiating parturition and oviposition or of oviductal innervation.

The proximate endocrine control of oviposition and parturition involving a neurohypophysial hormone [e.g. oxytocin, arginine vasotocin (AVT)] stimulating the local effects of prostaglandins (PG) is relatively well understood and similar across vertebrates. The relevant hormone in reptiles is AVT (Cree and Guillette 1991): in both oviparous and viviparous species AVT induces uterine contractions *in vitro*

(Guillette and Jones 1980; Mahmoud et al. 1987; Cree and Guillette 1991; Fergusson and Bradshaw 1992; Guillette et al. 1992), while exogenous AVT is able to stimulate oviposition (Ewert and Legler 1978; Guillette and Jones 1982) or parturition (Guillette 1979; Guillette et al. 1990; Cree and Guillette 1991) in a range of species.

The ability of neurohypophysial hormones to induce oviposition or parturition may be modulated by oviductal innervation. There is seasonal variation in adrenergic innervation of the oviduct in *Sceloporus jarrovi* (Rooney et al. 1997); while the presence of both α - and β -adrenergic receptors in the oviducts has been demonstrated in several lizard species via a series of *in vitro* experiments (Zurich et al. 1971; Jones et al. 1983; Cree and Guillette 1991). Arginine vasotocin therefore appears to be a more powerful stimulator of parturition *in vivo*, and more capable of overriding β -adrenergic inhibition than $\text{PGF}_{2\alpha}$ (Guillette et al. 1991). There is also an interaction between uterine innervation and the action of PGs in viviparous reptiles: in female *Hoplodactylus maculatus* pre-treatment with dichloroisoproterenol lifts the inhibition of the action of $\text{PGF}_{2\alpha}$, and isoproterenol blocks the *in vitro* contractions induced by $\text{PGF}_{2\alpha}$ but not AVT (Cree and Guillette 1991). *Anolis carolinensis* is apparently the only reptilian species that does not respond to *in vivo* injections of AVT: females do become responsive upon administration of a β -adrenergic agonist or surgical denervation of the uterus (Jones et al. 1983; Summers et al. 1985). Inhibition of oviductal contractions can also occur via sympathetic arousal of the nervous system (e.g. during stress) by stimulation of β receptors (Jones and Guillette 1982).

The most important proximate environmental factor controlling the timing of reproduction in reptilian species is temperature (Licht 1984), although its effects have rarely been tested experimentally. In the viviparous lizards *N. metallicus* and *N.*

microlepidotus AVT induced parturition more rapidly at warmer temperatures (22 and 28°C), while cooler temperatures (6 and 15°C) delayed, but did not prevent, parturition in response to AVT (Girling et al. 2002a). Indeed the ability of some viviparous squamates to delay parturition opportunistically for up to 4 weeks if environmental conditions are poor (N. Atkins et al. unpublished data - Ch. 3, 4; Swain and Jones 2000) may be mediated by an inhibition of the AVT response at cooler temperatures. In this paper, we investigate the endocrine and neural mechanisms that control parturition in viviparous squamates and the influence of temperature upon these mechanisms, using two related model species with different reproductive strategies.

Niveoscincus microlepidotus is an alpine species with biennial reproduction (Hutchinson et al. 1989; Olsson and Shine 1998, 1999). Embryonic development is complete in late autumn, but, although under laboratory conditions viable young may be born in autumn (Girling et al. 2002a, 2002b), in the field, parturition does not occur until late in the following spring. *Niveoscincus microlepidotus* therefore provides us with an ideal model species with which to investigate potential environmental mechanisms by which the endocrine control of parturition is modulated. We suggest that in autumn, environmental factors mediated via adrenergic innervation inhibit the endocrine cascade involved in parturition, with that inhibition being removed in spring. We therefore hypothesised that in *N. microlepidotus* *in vitro* uterine preparations would be responsive to AVT and $\text{PGF}_{2\alpha}$ in both autumn and spring, and that pre-treatment with the β -adrenergic agonist isoproterenol would decrease the effectiveness of AVT in stimulating uterine contractions.

In contrast, *Niveoscincus ocellatus* is an annually reproducing lizard (Jones et al. 1997). This species occurs over a wide altitudinal range (Rawlinson 1974), so for this study we selected a sub-alpine population as providing the best comparator for *N. microlepidotus*. Previous work on this species has shown that high altitude populations are less able to defer parturition in response to cold temperatures than low altitude populations (N. Atkins et al. unpublished data – Ch. 4). We investigated the potential for β -adrenergic or environmental (temperature) modulation of the response to exogenous AVT, through two experiments carried out in vivo: preliminary experiments indicated that it was not possible to reliably prepare in vitro uteri in this species. We hypothesised, first, that females treated with AVT would be less likely to give birth if pre-treated with the β -adrenergic agonist, isoproterenol. Second, we hypothesised that in these viviparous lizards [in contrast to the annually breeding *N. metallicus* and the biennial *N. microlepidotus* (Girling et al. 2002a)], the ability of exogenous AVT to induce parturition would not be significantly affected by temperature.

Methods

Experiment 1: *In vitro* response of isolated uteri of *N. microlepidotus* to AVT and $\text{PGF}_{2\alpha}$, and the effect of pre-treatment with isoproterenol on uterine contractions in response to AVT

Late pregnant females of *N. microlepidotus* were captured from the summit of Mt. Wellington (42°53'S, 147°14'E; 1,270 m above sea level). Twenty females were collected in autumn (March 2004), before hibernation (approximately 6-7 months

prior to the expected time of parturition), and another 20 females were collected in spring (October) of the same year at the expected time of natural parturition (Olsson and Shine 1998, 1999). On return to the laboratory, females were held in a large container, without either room lighting or a basking light (and therefore no food): even in March, females can give birth if provided with warm conditions (N. Atkins, personal observation). Water was provided *ad libitum*, and the experiment carried out within a week of capture.

Snout-vent length (SVL) and mass of each female were recorded. Animals were rapidly chilled and killed by decapitation and pithing, and the pregnant uterine (sensu Girling 2002) portion of each oviduct was removed. The embryo/embryos were expelled from the uterus by gentle finger pressure. For both groups of females, the embryos were viable, and the neonates were maintained in the laboratory until release into the field. The uteri were kept moist with Lizard Ringer's solution. Uteri that had held two embryos were prone to breakage, and preliminary experiments showed that only intact uteri responded to hormones. Thus only uteri previously containing a single embryo were used in these experiments, unfortunately reducing our sample sizes.

Uteri were mounted in an organ bath, connected to a force-displacement transducer (Harvard apparatus heart/smooth muscle transducer) and the trace was recorded using MacLab (V.3.3.6). Tension (0.3 g) was applied, to each freshly mounted uterus to maintain a degree of stretch similar to that observed in the pregnant oviduct. Uteri were maintained in Lizard Ringer's solution aerated with 95% O₂/5% CO₂ at 26°C: LaPointe (1977) demonstrated that in *in vitro* studies, maximal sensitivity of the uteri to AVT occurs at a similar temperature to the preferred body temperature of the species (*N. microlepidotus*: Melville 1998). The

uteri were allowed to equilibrate for 20 min. The bath was then flushed with Ringer's solution and the uteri exposed to one of the following regimes (the bath was flushed between each addition): pre-treatment 1 (10 min), pre-treatment 2 (10 min), treatment (30 min).

Group 1: Ringer's solution, Ringer's solution, Ringer's solution

Group 2: Ringer's solution, Ringer's solution, AVT

Group 3: Ringer's solution, Ringer's solution, $\text{PGF}_{2\alpha}$

Group 4: Ringer's solution, isoproterenol, AVT

(There were insufficient uteri available to repeat group 4 with $\text{PGF}_{2\alpha}$.)

Each addition was made in 0.5 ml of Ringer's solution and was added to the bath over the aerator. The concentrations were 100 ng/ml AVT (acetate salt, Sigma Chemical Company), 1 $\mu\text{g/ml}$ $\text{PGF}_{2\alpha}$ (Tris salt, Sigma Chemical Company), and 1 $\mu\text{g/ml}$ isoproterenol (hydrochloride, Sigma Chemical Company) (a β -adrenoreceptor agonist): these doses were derived from those used in the viviparous gecko *H. maculatus* (Cree and Guillette 1991). Where applicable, contralateral uteri were exposed to different treatments. The mean amplitude of the tonic contraction occurring in response to AVT (or $\text{PGF}_{2\alpha}$) was measured, and for groups 2 and 4 the mean tonic relaxation in response to isoproterenol was measured.

Experiment 2: Response to AVT and $\text{PGF}_{2\alpha}$ *in vivo* in *N. ocellatus*, and the effect of pre-treatment with isoproterenol on induction of parturition in response to AVT

Thirty-six pregnant females of *N. ocellatus* were captured from a sub-alpine population near Miena (41°98'S, 146°72'E), Tasmania (approximately 1,030 m above sea level). The females were collected in January, about 2 weeks before the

expected date of parturition (Wapstra et al. 1999). Animals were returned to the laboratory and treated for mites by dipping in Neguvon[®], with further dippings at regular fortnightly intervals (Atkins and Wapstra 2004 - App. 1). Females were maintained in pairs in plastic containers (30 cm X 20 cm X 10 cm), with mesh lids and paper pellets for bedding, in an air-conditioned room provided with a 11:13 h light:dark regime at natural light intensities and a background temperature set to 12°C. Each container included a basking area and a thermal gradient (12–35°C) was obtained through a low wattage (25 W) light bulb over one end of the cage (between 08:00 and 17:00 h). Lizards were fed with *Tenebrio* larvae (mealworms), meaty cat food or pear-banana baby food three times per week (except for the first 3 days of the experiment). A calcium supplement was dusted over their food at regular intervals and multi-vitamin supplements supplied in the drinking water, which was available *ad libitum*.

The experiment was started when the first female gave birth to a healthy live litter. The remaining pregnant females were allocated to one of six groups (N = 5 for group 1, remaining groups N = 6), and maintained under basking lights in the same room.

The treatment regimes, consisting of one pre-treatment and one treatment, were as follows:

Group 1: Ringer's solution, Ringer's solution

Group 2: Isoproterenol, Ringer's solution

Group 3: Ringer's solution, AVT

Group 4: Isoproterenol, AVT

Group 5: Ringer's solution, PGF_{2α}

The dose of AVT (acetate salt, Sigma Chemical Company) was 0.75 µg/g mean body weight (6 µg/150 µl) in Ringer's solution and was based on that which successfully induces parturition in *N. microlepidotus* and *N. metallicus* (Girling et al. 2002a), and an earlier pilot study on *N. ocellatus* (N. Atkins and S.M. Jones, unpublished data – App. 2). The dose of PGF_{2α} (Cloprostenol) was 2.5 µg/g mean body weight (20µg/100 µl) in Ringer's solution, based on a dose which induced parturition in *N. metallicus* (Bennett 1997), and an earlier pilot study on *N. ocellatus* (N. Atkins and S.M. Jones, unpublished data). The dose of isoproterenol was estimated from the literature regarding doses of dichlorisoproterenol (a β-adrenoreceptor blocker) and assuming a similar effect (Jones et al. 1983; Summers et al. 1985; Cree and Guillette 1991), and was determined to be 0.12 µg/g mean body weight (1 µg/50 µl) of isoproterenol (hydrochloride, Sigma Chemical Company) in Ringer's solution.

On the morning of the experiment, each female received an intraperitoneal injection of the appropriate pre-treatment followed by the treatment injection 20 min later, as in Cree and Guillette (1991). The time of injection was recorded, and all injections were given between 09:00 and 10:30 h. Females were then checked half-hourly for the first day until lights out, and then several times a day for the next 2 days: the time of parturition was recorded for each neonate. After the apparent completion of parturition, females and neonates were weighed and measured (SVL), and all females were palpated to determine whether they still retained embryos *in utero*.

Experiment 3: Effect of temperature on induction of parturition in *N. ocellatus* by exogenous AVT

Forty-one pregnant females of *N. ocellatus* were captured from a sub-alpine population from the Central Plateau (41°51'S, 146°34'E) of Tasmania (approximately 1,200 m above sea level). The females collected were about 2 weeks before the expected date of parturition (January) (Wapstra et al. 1999), and were housed as for Experiment 2.

The experiment was started when the first female gave birth to a healthy live litter. The remaining pregnant females were then allocated to one of eight groups ($N = 5$ for all groups) and moved to the appropriate temperature-controlled room at least 12 h before injection. Each female was housed individually in a small transparent plastic container with access to water. No cover or heat-lamp was provided so females could not thermoregulate, and room lighting was provided on a 9:15 h light:dark regime. Two groups of animals were allocated to each temperature: one treatment (AVT) and one control group. The room temperatures were 6, 15, 22 or 28°C, as in an earlier experiment investigating the effect of temperature on induction of parturition in *N. microlepidotus* and *N. metallicus* (Girling et al. 2002a).

Within each temperature, the treatment group received exogenous AVT in Lizard Ringer's solution ($6\text{ }\mu\text{g}/150\text{ }\mu\text{l} = 0.75\text{ }\mu\text{g/g}$ body weight), and the control group received Lizard Ringer's solution (150 μl). On the morning of the experiment, each female received an intraperitoneal injection of AVT or Lizard Ringer's solution and the time of the injection was recorded. All injections were given between 09:00 and 10:30 h. Females were then checked half-hourly for the first day (until lights out), and then several times a day for the next 2 days to determine whether parturition had occurred. After completion of parturition, females and neonates were weighed and measured (SVL), and all females were palpated to check that they had no remaining embryos *in utero*.

Statistics

All analyses were performed in SAS (v6.12). Data are presented as means \pm one standard error (SE). Homogeneity of group variances were assessed by studying plots of standard deviations against means, whereas assumptions of normality were checked by examining plots of standardized residuals against estimated values and the normal probability curves of the residuals; and the appropriate transformation to the data performed. For Experiment 1, differences between the amplitudes (log-transformed) of tonic contractions produced by AVT and $\text{PGF}_{2\alpha}$ were analysed by Analysis of Variance (ANOVA). For Experiments 2 and 3, the mean difference in time (min), from the treatment injection until the first birth between the treatment groups was analysed using ANOVA, with significant differences between treatment groups identified using a Tukey's test. For Experiments 2 and 3, differences in maternal mass between treatments were also assessed by ANOVA.

Results

Experiment 1: *In vitro* response of isolated uteri of *N. microlepidotus* to AVT and $\text{PGF}_{2\alpha}$, and the effect of pre-treatment with isoproterenol on uterine contractions in response to AVT

Isolated uteri of *N. microlepidotus* collected in both autumn and spring contracted in response to AVT, but were less responsive to $\text{PGF}_{2\alpha}$, and exhibited no response to treatment with Ringer's solution (group 1: controls). Examples of individual

responses (contraction and/or relaxation) of isolated uteri to AVT and to pre-treatment of isoproterenol followed by treatment with AVT are presented in Fig. 1.

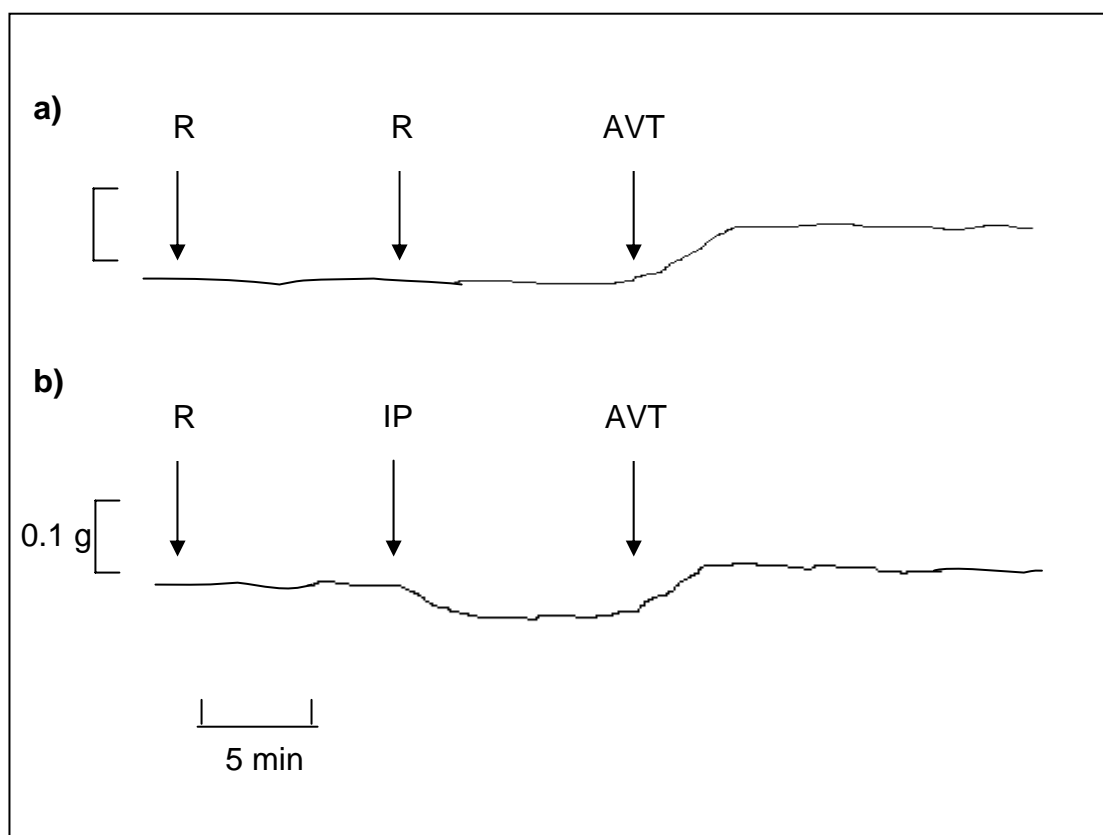


Fig. 1 Examples of contractile/relaxation responses of isolated uteri of *Niveoscincus microlepidotus* to **a** arginine vasotocin (AVT) after pre-treatment with Ringer's solution (R), and **b** response to AVT after pre-treatment with both Ringer's solution (R) and isoproterenol (IP). Scale bar for time (horizontal axis) = 5 min; scale bar for tension (vertical axis) = 0.1 g.

For *N. microlepidotus* females collected in autumn, all group 2 uteri treated with AVT responded with a large tonic contraction (Table 1). Uteri exposed to $\text{PGF}_{2\alpha}$ (group 3) also responded with a tonic contraction, which was generally less than the AVT induced contraction, but only half the uteri responded to $\text{PGF}_{2\alpha}$. Tonic contractions in response to AVT and $\text{PGF}_{2\alpha}$ were sustained for the 30 min of

Table 1 Effects of AVT and PGF_{2α} on isolated uteri of *Niveoscincus microlepidotus*, and effect of pre-treatment with isoproterenol on the response to AVT. A) Experiment in autumn B) Experiment in spring. Values are mean ± SE.

A)

Group	Pre-treatment 1	Pre-treatment 2	Amplitude of tonic relaxation (g)	Treatment	No. of uteri responding / total	Mean amplitude of tonic contraction (g)
1	Ringer's soln.	Ringer's soln.	~	Ringer's soln.	0/3	~
2	Ringer's soln.	Ringer's soln.	~	AVT	3/3	0.08 ± 0.04
3	Ringer's soln.	Ringer's soln.	~	PGF _{2α}	2/4	0.04
4	Ringer's soln.	Isoproterenol	0.01	AVT	1/1	0.13

B)

Group	Pre-treatment 1	Pre-treatment 2	Mean amplitude of tonic relaxation (g)	Treatment	No. of uteri responding / total	Mean amplitude of tonic contraction (g)
1	Ringer's soln.	Ringer's soln.	~	Ringer's soln.	0/4	~
2	Ringer's soln.	Ringer's soln.	~	AVT	4/6	0.04 ± 0.01
3	Ringer's soln.	Ringer's soln.	~	PGF _{2α}	1/5	0.02
4	Ringer's soln.	Isoproterenol	0.06 ± 0.01	AVT	4/6	0.07 ± .01

recording. In the single uterus that responded, exposure to isoproterenol during the second pre-treatment period (group 4) led to a pronounced tonic relaxation lasting the 10 min of recording. This relaxation did not, however, prevent a strong tonic contraction in response to AVT, which was sustained for the 30 min of recording.

For *N. microlepidotus* females collected in spring, exposure of the uteri to AVT (group 2) led to a pronounced tonic contraction: the majority of females responded (4/6), and the contractions were sustained for 30 min. No significant difference in response was detected, in response to AVT, for uteri collected in autumn ($F_{(1,7)} = 2.49$, $P = 0.16$, log transformed): spring uteri demonstrated a reduced response to $\text{PGF}_{2\alpha}$ (group 3): only one of the five uteri responded, and with a reduced tonic contraction. Pre-treatment with isoproterenol (group 4) led to a tonic relaxation that did not prevent contraction in response to AVT; the majority of females responded (4/6), but the amplitude of the contraction was reduced by half, in two of the four uteri, after 30 min. The mean peak amplitude did not differ significantly from that produced in AVT-treated group 2 uteri ($F_{(1,6)} = 2.92$, $P = 0.14$, log transformed).

Experiment 2: Response to AVT and $\text{PGF}_{2\alpha}$ *in vivo* in *N. ocellatus*, and the effect of pre-treatment with isoproterenol on induction of parturition in response to AVT

In *N. ocellatus*, parturition was induced in response to an injection of either AVT or $\text{PGF}_{2\alpha}$, and the response to AVT was modulated by pre-treatment with isoproterenol (Table 2). No control females (group 1) gave birth in response to injection with Ringer's solution. Although two births occurred in group 2, females pre-treated with isoproterenol, the mean time to first birth was greater than 4 h. There was no

Table 2 Responses to AVT and PGF_{2α} in late pregnant *Niveoscincus ocellatus*, and the effect of pre-treatment with isoproterenol on the response to AVT: the proportion of females that gave birth, and the mean (± SE) time to first birth within the group are presented.

Group	Pre-treatment	Treatment	No. of females responding / total	Time to first birth (min)
1	Ringer's soln.	Ringer's soln.	0/5	~
2	Isoproterenol	Ringer's soln.	2/6	> 240
3	Ringer's soln.	AVT	6/6	54 ± 7
4	Isoproterenol	AVT	4/6	132 ± 20
5	Ringer's soln.	PGF _{2α}	5/6	88 ± 6

significant difference in maternal mass between the groups in which there were births ($F_{(3,20)} = 0.05$, $P = 0.99$, log transformed).

All females responded to AVT following pre-treatment with Ringer's solution (group 3), and four out of six females responded to AVT following pre-treatment with isoproterenol (group 4). The time until first birth was significantly influenced by treatment ($F_{(2,12)} = 12.91$, $P = 0.001$): pre-treatment with isoproterenol significantly increased the response time to AVT ($P < 0.05$).

In group 5, five females out of six responded, giving birth to their entire litter. The response time to $\text{PGF}_{2\alpha}$ was significantly shorter than the response to AVT after pre-treatment with isoproterenol ($P < 0.05$), but not different to the treatment of only AVT ($P > 0.05$).

Only two viable litters were produced during this experiment, by females from groups 2 and 5. The other control female (group 2) and all experimental females gave birth to low-viability litters (defined as death on or the day after birth). Nine (out of 16) mothers consumed their neonates (observed or inferred by the presence of afterbirth), and a further two litters including one neonate dead at birth; all neonates were at stage 39 or 40 (Dufaure and Hubert 1961). The mean mass and SVL of the single viable litter produced by an experimentally treated female, were 536.0 mg and 29.49 mm, respectively. Mean mass and SVL for neonates from the low-viability litters were less than the viable litter, being 405.6 ± 57.1 mg (11) and 26.83 ± 1.11 mm (11), respectively, suggesting that we had induced premature parturition.

Experiment 3: Effect of temperature on induction of parturition in *N. ocellatus* by exogenous AVT

All pregnant females receiving AVT gave birth to between one and five young. In the AVT- treated groups, the time until first birth was significantly longer as the treatment temperature decreased ($F_{(3,16)} = 143.45$, $P = 0.0001$, log transformed) (Fig. 2), and delivery of a full clutch was less likely at lower temperatures: at the completion of the experiment (58 h after initial injection), all females in the 6°C AVT group, one female in the 22°C AVT group, and two females in the 28°C AVT group retained one or two live embryos *in utero*. Females (at the two higher temperatures) with larger litters were less likely to deliver their entire litter in response to AVT.

Among the control (Ringer's solution injected) females, only one (housed at 22°C) gave birth during the experiment. There was no significant difference in maternal mass between the AVT and control groups of females ($F_{(3,16)} = 0.49$, $P = 0.69$, log transformed).

The mean mass and SVL per litter of viable experimental neonates, were 586.1 ± 17.8 mg (7) and 29.59 ± 0.25 mm (7), respectively. Mean mass and SVL of low-viability experimental litters were less than the viable litters, and were 529.5 ± 13.1 mg (8) and 28.82 ± 0.41 mm (8) respectively (neonates at stage 39 or 40). The remaining five litters included one or more dead neonates/stillborns. One female held at 28 °C ate all her low-viability neonates, and one female from each of the 6 and 15°C groups ate one of their neonates each from a low-viability litter of three (observed or inferred by the presence of afterbirth).

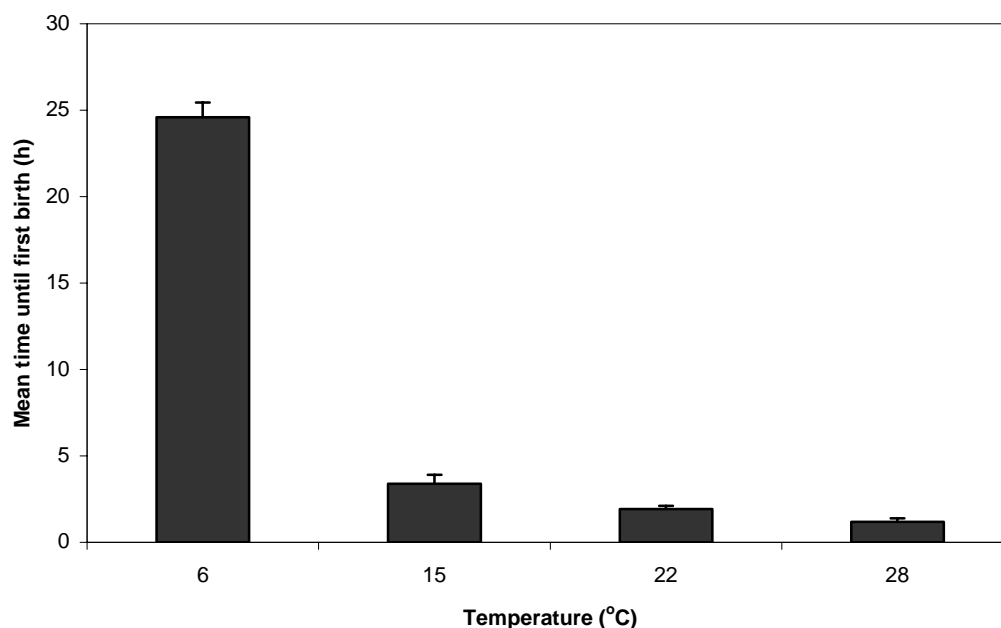


Fig. 2 Mean time until birth of first neonate after an injection of AVT in *N. ocellatus* housed at different temperatures, with a sample size of 5 for each temperature. *Error bars* are one standard error of the mean (SE).

Discussion

Isolated uteri of *N. microlepidotus* contracted in response to both AVT and $\text{PGF}_{2\alpha}$ confirming that, as in other reptiles (Guillette et al. 1991), both hormones are involved in the process of parturition in this species. In *S. jarrovi*, for example, AVT alone or in conjunction with arachidonic acid (AA, a prostaglandin precursor) stimulates rapid “birth” in isolated oviducts (Guillette et al. 1992). In *N. microlepidotus*, the isolated uteri were less responsive to $\text{PGF}_{2\alpha}$ than to AVT. Although we did not carry out a dose-response experiment, a similar dose of $\text{PGF}_{2\alpha}$ induced tonic contractions in isolated uteri of *H. maculatus* (Cree and Guillette 1991)

whereas the same dose of $\text{PGF}_{2\alpha}$ or of PGE_2 stimulated 60–80% of total available “births” in *S. jarrovi* (Guillette et al. 1992).

Our results confirm the potential for β -adrenergic modulation of the endocrine cascade leading to parturition in *N. microlepidotus*: β -adrenergic stimulation (isoproterenol), while not preventing a response, affected the duration of AVT-induced tonic contractions. Similarly, in *A. carolinensis*, both isoproterenol and epinephrine inhibited the amplitude and duration of AVT-induced tonic contractions in isolated uteri (Jones et al. 1983). However, in *H. maculatus*, β -adrenergic stimulation with isoproterenol inhibited the uterotonic response to $\text{PGF}_{2\alpha}$, but not to AVT (Cree and Guillette 1991), leading those authors to suggest that in *H. maculatus* AVT and $\text{PGF}_{2\alpha}$ may induce oviductal contractions by different mechanisms.

Our hypothesis that uterine preparations of *N. microlepidotus* would be responsive to exogenous hormones in both autumn and spring was supported: there was no significant difference between autumn and spring in the degree of response of isolated uteri to AVT, but there was some evidence that responsiveness to $\text{PGF}_{2\alpha}$ was lower in spring. Comparisons of uteri from reproductively inactive and active *A. carolinensis* found that while the responses to AVT were qualitatively similar, season greatly influenced the magnitude of response (Jones et al. 1987). However, Girling et al. (2002a) have demonstrated that *N. microlepidotus* females collected in autumn will give birth if injected with AVT, and in the biennially reproducing *H. maculatus* responsiveness to hormonal stimulation of parturition by AVT did not differ between autumn and spring (Rock 2005). Girling et al. (2002a) hypothesised that in *N. microlepidotus* decreasing temperatures act at some level within the brain, preventing or slowing the secretion of AVT in autumn. Our results demonstrate conclusively that natural births in autumn are not prevented by down regulation of

receptors for AVT or prostaglandin: the oviducts are clearly capable of responding to exogenous hormone *in vitro*, suggesting that parturition is inhibited by mechanisms acting higher up the endocrine cascade.

For *N. ocellatus*, the *in vivo* experiment (Experiment 2) illustrated that either exogenous AVT or PGF_{2α} can stimulate parturition. The high incidence of stillborn embryos in both Experiments 2 and 3 presumably reflected the inevitable spread in degree of completion of embryonic development at the time of the experiment (also evidenced by the smaller masses and sizes of the low-viability litters); in the natural population, births are spread over about 3-4 weeks (N. Atkins et al. unpublished data - Ch. 4; Wapstra et al. 1999). Girling et al. (2002a) also noted a high incidence of stillbirths in their experiment using *N. metallicus*. Induction of oviposition in the tuatara results in smaller eggs, than those laid naturally, which results in significantly smaller hatchlings following incubation (Nelson et al. 2004). Like Bennett (1997) and Girling et al. (2002a), we noted that many females ate their stillborn offspring, presumably as a mechanism for maternal recycling of nutrients (Blackburn 1998; Lourdaïs et al. 2005).

Guillette et al. (1991) reviewed the evidence regarding the role of prostaglandins in parturient behaviour and oviposition in reptiles. They hypothesised that, as in mammals and birds, rising plasma AVT stimulates PG synthesis by the oviduct, and that PG stimulates oviductal contractions via a paracrine effect. This evidence suggests that late pregnant females should respond more readily to exogenous PG than to AVT. However, in *N. ocellatus*, females were more responsive to AVT than to PGF_{2α}, at least at the doses we used. In late-pregnant *S. jarrovi*, a large range of doses of PGF_{2α} (25, 50, 100 ng/g, 1 µg/g) initiated birth in between 65 and 85% of females and in a threshold-dependent fashion, with a dose of 10 ng/g

initiating no births (Guillette et al. 1992). Females of *H. maculatus* are also less responsive to PGF_{2α} (2 µg/g) than to AVT, although four/five females responded to PGF_{2α} if pre-treated with the β-adrenoreceptor blocker dichloroisoproterenol (Cree and Guillette 1991), again illustrating the potential for inter-species differences in the interactions between the endocrine and adrenergic systems controlling parturition in viviparous squamates.

Our hypothesis that pre-treatment with the β-adrenergic agonist isoproterenol would prevent or slow induction of parturition by AVT in *N. ocellatus* was supported. It is possible that a shorter, or longer, time interval (we used 20 min) between the administration of the isoproterenol and AVT would have resulted in prevention of the induction of parturition. In *A. carolinensis*, the timing of the administration of dichloroisoproterenol to allow the action of AVT in induction of oviposition is important, a 15 min interval being successful and 30 or 5 min intervals unsuccessful (Summers et al. 1985). There have been no other studies of the interaction *in vivo* between isoproterenol and AVT in reptiles. In hens, isoproterenol does not delay oviposition (Wechsung and Houvenaghel 1987), but adrenalin (both an α- and a β-adrenergic drug) does delay oviposition, inducing both a relaxation of the uterus (β-effect) and, at the same time a contraction of the vagina (α-effect) (Crossley 1983; Hughes and Gilbert 1984). In reptiles, the β-adrenergic antagonist dichloroisoproterenol has a permissive effect on the induction of oviposition in *A. carolinensis* (Summers et al. 1985), but does not enhance the effect of AVT in *H. maculatus* (Cree and Guillette 1991)

It appears, then, that the adrenergic system modulates uterine responsiveness in our two study species and in other reptiles. Indeed, Guillette et al. (1991) hypothesised that in viviparous reptiles parturition is potentiated by removal of a β-

adrenergic block on oviductal contraction. Is this a route whereby environmental conditions may influence the endocrine control of parturition, and thus permit deferral of birth in adverse conditions?

Temperature clearly does influence the response of *N. ocellatus* to exogenous AVT, even in this high altitude population. As in *N. metallicus* and *N. microlepidotus* (Girling et al. 2002a), the cooler temperatures significantly delayed, but did not completely inhibit parturition. However, unlike females of the comparator species, females held at 15°C still gave birth within a relatively short interval (~ 3 h versus ~ 8–10 h). This suggests that inhibition of parturition by cooler temperature is less marked in *N. ocellatus*, which may explain their reduced ability to defer parturition. The short intervals to first birth in response to exogenous AVT in females held at 22 and 28°C presumably reflect maximal sensitivity of the oviductal tissue to AVT near the species' preferred body temperature (LaPointe 1977). None of the females held at 6°C delivered an entire litter, which may reflect the short half-life of AVT *in vivo* (Harding and Rowe 2003), in comparison with the extended reaction time at this temperature. It may also reflect temperature effects on enzyme kinetics in the muscle or blood flow to the uterus limiting delivery of the active compound to the myometrium in these ectothermic animals. However, injection of AVT at 150 or 1500 ng/g did not induce birth of complete litters in any female of *H. maculatus* held at a temperature appropriate to that species, and only 50% of females responded, although the authors suggested that the higher dose is certainly pharmacological (Cree and Guillette 1991). Several female *N. ocellatus* with larger litters did not give birth to their entire litter at the higher temperatures, but this probably reflects the relatively rapid clearance of AVT from the body following the single treatment dose.

Thus, our experiments provide further support for a role for β -adrenergic innervation in the control of parturition in viviparous reptiles. In the biennially reproducing *N. microlepidotus*, it is clear that the endocrine mechanism necessary for oviductal responses to neurohypothalamic stimulation is in place in autumn (at the completion of embryonic development) as well as in spring (at the time of natural parturition). In the annually reproducing *N. ocellatus*, and in *N. microlepidotus* and *N. metallicus* (Girling et al. 2002a), environmental conditions, specifically, temperature, clearly modulate the response of reptilian oviducts to AVT *in vivo*. We therefore suggest that inhibition of contractility by β -adrenergic neurones may represent a mechanism whereby parturition is deferred until environmental conditions are conducive to neonatal survival. It is also possible that environmental influences may be mediated via the hypothalamo-pituitary-adrenal axis. In mice, for example, parturition is delayed by stress acting via adrenergic mechanisms (Douglas et al. 2002), while stimulation of the sympathetic nervous system by short-term stress can inhibit parturition in *H. maculatus* (Cree and Guillette 1991) and oviposition in *A. carolinensis* (Jones et al. 1983; Summers et al. 1985). Our results suggest further investigation of the interactions between adrenergic innervation and environmental stimuli in modulating oviductal contractility and in potentiating deferral of parturition in viviparous lizards is required.

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References

- Andrews RM, Rose BR (1994) Evolution of viviparity: constraints on egg retention. *Physiol Zool* 67:1006-1024
- Atkins N, Wapstra E (2004) Successful treatment of a mite infestation in gravid spotted snow skinks (*Niveoscincus ocellatus*). *Herpetofauna* 34:66-69
- Bennett EJ (1997) A study of gestation in the viviparous skink, *Niveoscincus metallicus*. Honours thesis, School of Zoology. University of Tasmania
- Blackburn D (1998) Resorption of oviductal eggs and embryos in squamate reptiles. *Herpetol J* 8:65-71
- Cree A, Guillelte JL Jr (1991) Effect of β -adrenergic stimulation on uterine contraction in response to arginine vasotocin and prostaglandin $\text{F}_{2\alpha}$ in the gecko *Hoplodactylus maculatus*. *Biol Reprod* 44:499-510
- Crossley JC (1983) Prevention of epinephrine and stress-induced egg-laying delay by feeding propranolol to the laying hen. *Poult Sci* 62:375-378
- Douglas AJ, Leng G, Russell JA (2002) The importance of oxytocin mechanisms in the control of mouse parturition. *Reproduction* 123:543-552

-
- Dufaure JP, Hubert J (1961) Table de development du lezard vivipare: *Lacerta (Zootoca) vivipara* Jaquin. Arch d'Anat Micro Morphol Exp 50:309-328
- Ewert MA, Legler JM (1978) Hormonal induction of oviposition in turtles. Herpetologica 34:314-319
- Fergusson B, Bradshaw SD (1992) *In vitro* uterine contractions in the viviparous lizard *Tiliqua rugosa*: effects of gestation and steroid pretreatment *in vivo*. Gen Comp Endocrinol 86:203-210
- Girling JE (2002) The reptilian oviduct: a review of structure and function and directions for future research. J Exp Zool 293:141-170
- Girling JE, Jones SM, Swain R (2002a) Induction of parturition in snow skinks: can low temperatures inhibit the actions of AVT? J Exp Zool 293:525-531
- Girling JE, Jones SM, Swain R (2002b) Delayed ovulation and parturition in a viviparous alpine lizard (*Niveoscincus microlepidotus*): morphological data and plasma steroid concentrations. Reprod Fertil Dev 14:43-53
- Guillette JL Jr (1979) Stimulation of parturition in a viviparous lizard (*Sceloporus jarrovi*) by arginine vasotocin. Gen Comp Endocrinol 38:457-460
- Guillette JL Jr, Jones RE (1980) Arginine vasotocin-induced *in vitro* oviductal contractions in *Anolis carolinensis*: effects of steroid hormone pretreatment *in vivo*. J Exp Zool 212:147-152
- Guillette JL Jr, Jones RE (1982) Further observations on arginine vasotocin-induced oviposition and parturition in lizards. Herpetol 16:140-144
- Guillette JL Jr, Jones RE, Fitzgerald KT, Smith H (1980) Evolution of viviparity in the lizard genus *Sceloporus*. Herpetologica 36:201-215

- Guillette JL Jr, Hensley AP, Matter JM, Jaffe PH (1990) Indomethacin influences arginine vasotocin-induced parturition and oviposition in lizards (*Sceloporus jarrovi* and *Sceloporus undulatus*). *Theriogenology* 33:809-818
- Guillette JL Jr, Dubois DH, Cree A (1991) Prostaglandins, oviductal function, and parturient behaviour in nonmammalian vertebrates. *Am J Physiol* 260:R854-R861
- Guillette JL Jr, DeMarco V, Palmer B, Masson GR (1992) Effects of arachidonic acid, prostaglandin F_{2α}, prostaglandin E₂, and arginine vasotocin on induction of birth *in vivo* and *in vitro* in a viviparous lizard (*Sceloporus jarrovi*). *Gen Comp Endocrinol* 85:477-485
- Harding CF, Rowe SA (2003) Vasotocin treatment inhibits courtship in male zebra finches; concomitant androgen treatment inhibits this effect. *Horm Behav* 44:413-418
- Hughes BO, Gilbert AB (1984) Induction of egg shell abnormalities in domestic fowls by administration of adrenaline. *IRCS Med Sci Lib Comp* 19:969-970
- Hutchinson MN, Robertson P, Rawlinson PA (1989) Redescription and ecology of the two endemic Tasmanian scincid lizards *Leiopisma microlepidotum* and *L. pretiosum*. *Pap Proc R Soc Tas* 123:257-274
- Jones RE, Guillette JL Jr (1982) Hormonal control of oviposition and parturition in lizards. *Herpetologica* 38:80-93
- Jones RE, Summers CH, Lopez KH (1983) Adrenergic inhibition of uterine contractions and oviposition in the lizard *Anolis carolinensis*. *Gen Comp Endocrinol* 51:77 - 83

- Jones RE, Lopez KH, Summers CH, Austin HB (1987) Seasonal changes in the effects of arginine vasotocin and stretch on *Anolis* uterine contractions *in vitro*. *J Exp Zool* 242:233-239
- Jones SM, Wapstra E, Swain R (1997) Asynchronous male and female gonadal cycles and plasma steroid concentrations in a viviparous lizard, *Niveoscincus ocellatus* (Scincidae), from Tasmania. *Gen Comp Endocrinol* 108:271-281
- LaPointe JL (1977) Comparative physiology of neurohypophysial hormone action on the vertebrate oviduct-uterus. *Am Zool* 17:763-773
- Licht P (1984) Reptiles. In: Lamming GE (ed) *Marshall's physiology of reproduction*, vol I, 4th edn. Churchill Livingstone, Edinburgh, pp 206-282
- Lourdais O, Brischoux F, Shine R, Bonnet X (2005) Adaptive maternal cannibalism in snakes (*Epicrates cenchria maurus*, Boidae). *Biol J Linn Soc* 84:767-774
- Mahmoud IY, Cyrus RV, Wright DL (1987) The effect of arginine vasotocin and ovarian steroids on uterine contractility in the snapping turtle, *Chelydra serpentina*. *Comp Biochem Physiol* 86A:559-564
- Melville J (1998) The evolution of locomotory mode in the lizard genus *Niveoscincus*: an ecomorphological analysis of ecology, behaviour, morphology and performance ability. PhD thesis, School of Zoology. University of Tasmania
- Nelson NJ, Thompson MB, Pledger S, Keall SN, Daugherty CH (2004) Induction of oviposition produces smaller eggs in tuatara (*Sphenodon punctatus*). *N Z J Zool* 31:283-289
- Olsson M, Shine R (1998) Timing of parturition as a maternal care tactic in an alpine lizard species. *Evolution* 52:1861-1864

-
- Olsson M, Shine R (1999) Plasticity of frequency of reproduction in an alpine lizard, *Niveoscincus microlepidotus*. *Copeia* 1999:794-796
- Rawlinson PA (1974) Biogeography and ecology of the reptiles of Tasmania and the Bass Strait area. In: Williams WD (ed) Biogeography and ecology in Tasmania. Junk, W., The Hague, pp 291-388
- Rock J (2005) Delayed parturition: constraint or coping mechanism in a viviparous gekkonid? *J Zool Lond* 268:355-360
- Rooney AA, Donald JA, Guillette JL Jr (1997) Adrenergic and peptidergic innervation of the oviduct of *Sceloporus jarrovi* during the reproductive cycle. *J Exp Zool* 278:45-52
- Shanbhag BA, Saidapur SK, Radder RS (2003) Lowering body temperature induces embryonic diapause during prolonged egg retention in the lizard, *Calotes versicolor*. *Naturwissenschaften* 90:33-35
- Summers CH, Austin HB, Jones RE (1985) Induction of oviposition in cycling *Anolis carolinensis* requires an adrenergic receptor blocker and a high dosage of arginine vasotocin. *Gen Comp Endocrinol* 57:389-392
- Swain R, Jones SM (2000) Facultative placentotrophy: half-way house or strategic solution? *Comp Biochem Physiol* 127A:441-451
- Wapstra E, Swain R, Jones SM, O'Reilly J (1999) Geographic and annual variation in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Aust J Zool* 47:539-550
- Wechsung E, Houvenaghel A (1987) Influence of carbachol and isoproterenol on myoelectrical activity of the uterus, vagina, and duodenum in the conscious domestic hen. *Biol Reprod* 37:1108-1113

Zurich L, Paz de la Vega-Lemus Y, Lemus D (1971) Presence of adrenergic receptors in the uterus of two species of lizards, *Liolaemus gravenhorti* and *Liolaemus tenuis tenuis*. Biol Reprod 5:123-126

Part 3.

*Genetic control of offspring fitness –
paternal influence.*

Chapter 6a

Home range, multiple paternity and reproductive success in a viviparous squamate, *Niveoscincus ocellatus* (Gray 1845)

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Abstract

Utilising a well studied population of the viviparous skink, *Niveoscincus ocellatus*, potential mates and home range size for each individual were identified in a study spanning both mating periods (autumn and spring) of the species. With the accurate determination of paternity in squamate reptiles now commonplace, the degree of multiple paternity and the correlation of male characteristics and reproductive success can also be investigated. There was no intersexual difference in home range size and, with high numbers of overlapping home ranges between the sexes, there were many mating opportunities for females. Home range overlap was not, however, indicative of paternity, with only 26% of sires identified using this method; and furthermore, proximity distance was not useful in identifying sires. We were able to determine the paternity of 65% of offspring, and we confirmed that females mate successfully with multiple males: 93% of litters had multiple paternities. The number of recorded copulations for an individual female had no effect on offspring

characteristics at birth. While female size was correlated with litter size, no measured male characteristic was correlated with male reproductive success.

Introduction

Squamate (lizards, snakes and amphisbaenians) reptiles rarely exhibit parental care (O'Connor & Shine, 2004; Somma, 2003) and therefore the provision of genes at mating time is the only contribution of the male towards its offspring. One of the primary determinants of male mating success is body size, although territory/home range attributes, 'contest attributes', and alternative mating tactics may also contribute (Olsson & Madsen, 1998; Tregenza & Wedell, 2000). Females frequently mate promiscuously in squamates (Olsson & Madsen, 2001), suggesting that postcopulatory mechanisms to increase the probability of fertilisation [e.g., mate guarding (Olsson, Gullberg & Tegelstrom, 1996a), penis modifications or copulatory plugs (reviewed in Olsson & Madsen, 1998)] are under selection. Paternity may also be decided through competition of sperm in the female uterine tract or through cryptic female choice (Birkhead & Moller, 1992; Eberhard, 1998; Smith, 1984): this may be a common feature of lizard mating systems (Gullberg, Olsson & Tegelstrom, 1997; Olsson & Madsen, 1998; Olsson & Madsen, 2001; Richard *et al.*, 2005).

There is a high occurrence of both multiple matings and multiple paternity in reptile species (e.g. Gullberg *et al.*, 1997; Laloi *et al.*, 2004; Olsson & Madsen, 1998; Pearse, Janzen & Avise, 2002; Uller & Olsson, 2005). Furthermore, numerous studies, not only on reptiles, have demonstrated that females mated to multiple males have offspring with higher viability (Jennions & Petrie, 2000; Madsen *et al.*, 1992;

Olsson *et al.*, 1994; reviewed in Tregenza & Wedell, 2000), providing evidence of a role for genetic compatibility in mating outcomes (Jennions & Petrie, 2000).

However, both female common lizards (*Lacerta vivipara*) and female sand lizards (*L. agilis*) may mate multiple times partly due to sperm limitations and to avoid infertile males (Olsson & Shine, 1997; Uller & Olsson, 2005).

Before molecular techniques became the norm for determining paternity, male lizard reproductive success was generally estimated from measures of home range overlap with females or behavioural interactions because copulations are seldom observed (Cooper & Vitt, 1993; M'Closkey, Deslippe & Szpak, 1990; Olsson & Shine, 1996; Ruby, 1984). However, recent behavioural and genetic evaluations utilising molecular techniques of paternity suggest that this may be inaccurate (Abell, 1997; Bull, Cooper & Baghurst, 1998; Gardner *et al.*, 1999; Gullberg *et al.*, 1997; Hughes, 1998; Lebas, 2001; Lewis, Tirado & Sepulveda, 2000; Olsson *et al.*, 1996b). However, four studies that have analysed paternity using molecular markers in lizard species with non-territorial mating systems have concluded that paternity is highly indicated by proximity to a female (Abell, 1997; Bull *et al.*, 1998; Gullberg *et al.*, 1997; Lewis *et al.*, 2000).

We examined correlates of male reproductive success in the viviparous spotted snow skink, *Niveoscincus ocellatus*. Potential mates available to females were identified by home range overlap and proximity to females. The number of copulations, and therefore an indication of the number of possible mates, can easily be identified by the mating scars evident on the females at the end of the mating season in *N. ocellatus* (Jones, Wapstra & Swain, 1997). We already know that this species mates multiple times (Jones *et al.*, 1997; Wapstra *et al.*, 1999), but we have no idea whether this translates to multiple paternity. The aims of this present study

were to use molecular techniques (microsatellites) to 1) determine the degree of multiple paternity in our population and 2) identify predictors of male reproductive success.

Methods

Niveoscincus ocellatus is an annually breeding viviparous skink (Jones *et al.*, 1997; Wapstra & Swain, 2001a; Wapstra *et al.*, 1999). All sexually mature females mate in autumn, with females and males both storing sperm over winter; a proportion of the population experiences a second mating period in the spring before ovulation (Jones *et al.*, 1997). The species occupies a wide altitudinal range of Tasmania, Australia (Wapstra & Swain, 2001a): this study was conducted on a low elevation population of *N. ocellatus* near Orford (42°34'S, 147°52'E) on the east coast (50-75 m above sea level) of Tasmania. This site is typical of much of the coastal, cool temperate region of Tasmania (Rawlinson, 1974). Within this region, lizards are commonly active from the beginning of the austral spring (September) to the late austral autumn (mid-May), with occasional emergences over winter (Wapstra & Swain, 1996).

Reproductive and life history traits of this population have been documented previously (Jones *et al.*, 1997; Wapstra & Swain, 2001a; Wapstra & Swain, 2001b; Wapstra *et al.*, 1999; Wapstra, Swain & O'Reilly, 2001), and further details of the study site are described therein.

Home range

In the present study, lizards were caught using mealworm “fishing”, noosing with long rods, or by hand. Every adult *N. ocellatus* observed in the study area was

captured during February 2003 – December 2003, a time period encompassing both mating periods. Animals were marked permanently by toe clipping (if not already marked) and semi-permanently with individually numbered cloth tape (Tesa[®], Germany), for visual identification. Natural toe loss is observed in this population, and toe clipping does not affect behaviour and fitness in other species (Dodd, 1993; Hudson, 1995; Ott & Scott, 1999) or sprint speed in this species (Wapstra, unpublished data). At first capture, all lizards were weighed (to the nearest 0.1 g) using a field balance; and snout vent length (SVL), tail length, distance from vent to tail break (to the nearest mm) and head length and head width in the males measured. Sex was determined by hemipenes eversion (Wapstra *et al.*, 2004). A measure of body condition was obtained for the males by generating the residuals from a mass - SVL regression ($F_{(1,49)} = 76.15$, $P = 0.0001$, $R^2 = 0.61$). In males, the distal one cm portion of the tail was removed and stored in 90% ethanol for genotyping; tail autotomy and regeneration are common in snow skinks (Chapple, McCoull & Swain, 2002). By the end of the study, we had identified and marked 58 males and 74 females.

Data on home range size were collected by scan sampling, which involved walking slow random transects through the study area ($\sim 5000 \text{ m}^2$) and determining the location of marked lizards with a GPS unit. We also noted male and female lizards sighted at the same time. Observation time was equally distributed through the study area and the census route was varied during different sessions to minimise any potential bias in the number of sightings per individual. Lizards were recaptured only as required to replace their cloth tape marks. We surveyed the study area between February – June (before the austral winter, including the first mating period) and August – September (after the austral winter, including the second mating

period), usually in both the morning and the afternoon (majority of sightings by NA & EW). Two independent sightings of an individual on each day, one in the morning and one in the afternoon, were the maximum recorded for any individual. During the first census period (February – June), the site was visited on most days that weather permitted lizard activity - on average three times per week; after winter, in the second census period, the site was visited on average two times per week. Towards the end of the second census period, sighted females were captured and the number of copulatory scars (Jones *et al.*, 1997) was recorded. In late December, all gravid females in the final stages of gestation (including any females caught on the periphery of our study site) were collected and returned to the laboratory for birth; with their location noted upon capture. Incidental male sightings were recorded, and all non-gravid females were captured to assess reproductive condition.

We estimated the home range size of each lizard using the minimum convex polygon method (Ranges V software, Kenward & Hodder, 1996), using all the locations where the lizard was sighted. This method was used because of our interest in spatial arrangements between and within the sexes (Haenel, Smith & John-Alder, 2003; Rose, 1982). The number of times an individual is sighted can influence estimates of its home range size (Rose, 1982). We plotted cumulative home range size against the number of sightings, and the minimum number of sightings required was determined by the number of points needed to describe 80% of ultimate home range (Rose, 1982; Stone & Baird, 2002). Only individuals with the minimum number of sightings were used for overlap analyses, and lizards with fewer than the minimum number of sightings were included only as individuals that could be overlapped by the focal lizard.

Laboratory births

The gravid females were maintained in plastic terraria (30 cm x 20 cm x 10 cm), with mesh lids provided with cover and basking surfaces, in an air-conditioned room with a 14:10 h L:D cycle at natural light intensities. Under these standard conditions, the ambient temperature was 14 °C; a thermal gradient (14 – 35 °C) was obtained through a 25 W spot light (12 h/day) over one end of the cage. The lizards were fed with *Tenebrio* larvae (mealworms) and crushed fruit three times per week. A calcium and vitamin supplement was dusted over their food at regular intervals and drinking water was available *ad libitum*. Terraria were checked at least three times daily for newborns.

At birth, maternal length (SVL) and mass were recorded and a terminal tail sample (1 cm) was collected from the mother. All offspring were measured (mass, SVL), toe-clipped for identification and a tail sample (0.5 cm) was collected for genotyping. Sprint speed was measured (on the day of birth) using a computerised racetrack with five sets of photoelectric detectors: animals were heated to 28 °C (Melville, 1998) for 30 min and were run once down a 2 m track, with the fastest speed (of four 50 cm sections within the track) being used for analysis as a measure of “best performance” (Wapstra & O'Reilly, 2001).

Paternity analysis

All offspring, their mothers, and all potential sires, were genotyped for five polymorphic microsatellite loci, specially developed for this species (Wapstra *et al.*, unpublished data). Genomic DNA was extracted from tail tissue using a modified CTAB extraction procedure. Approximately 100 mg of homogenised tail tissue was incubated for 2 h at 65 °C in a 1.5 ml micro centrifuge tube containing 500 µl of

CTAB buffer (0.1 M Tris-HCl pH 8.0; 0.02 M EDTA; 1.4 M NaCl and 0.05M CTAB (hexadecyltrimethylammonium bromide)) and 5 µl of Proteinase K (20 mg/ml). The homogenate was extracted with 500 µl of chloroform-isoamyl alcohol (24:1) followed by a further extraction with phenol/chloroform-isoamyl alcohol (25:24:1), before a final extraction with chloroform-isoamyl alcohol prior to precipitation in isopropanol. DNA pellets were washed in 200µl of 70% ETOH, dried under vacuum and resuspended in 100 µl of deionised water.

The five microsatellite loci were amplified from the extracted genomic DNA using PCR (Wapstra *et al.*, unpublished data). Microsatellite alleles were separated and sized using a Beckman Coulter CEQ 8000 DNA Analysis System (Beckman Coulter, Fullerton CA). Alleles for each of the five loci were assigned by eye using the CEQ 8000 fragment analysis software.

Paternity was assigned using the CERVUS program (Marshall *et al.*, 1998). Sires were assigned paternity only if there were no genetic mismatches, and only a single father was assigned to each offspring.

Statistical analyses

Data are presented as means \pm standard errors. All analyses were performed in SAS (v 6.12). Determinants of male reproductive success (number of offspring sired) were identified using multiple regression. All other analyses were linear regressions or ANOVAs, unless stated. Where necessary, data were log-transformed to stabilise variances and/or normalise data: homogeneity of group variances was assessed by studying a plot of standard deviations against means, while assumptions of normality were checked by examining plots of standardized residuals against estimated values and the normal probability curve of the residuals.

Results

Home range

We obtained 390 observations of the 58 males identified as part of our study (four males were caught for the first time after winter). The minimum number of sightings required to establish male home range was seven: we were therefore able to generate home ranges for 25 of the 58 males identified. There was no significant difference ($F_{(1,56)} = 0.87$, $P = 0.35$, log transformed) in SVL between males with (63.40 ± 0.78 , $N = 25$) and without (64.27 ± 0.58 , $N = 33$) a generated home range. The mean home range size was $217 \pm 20 \text{ m}^2$ ($58 - 400 \text{ m}^2$, $N = 25$). The mean number of females overlapping a male's home range was 11 (range of 5-19), and the mean intrasexual overlap was 8.5 males (range of 2 – 15). In males, home range size was not significantly correlated with either SVL ($F_{(1, 23)} = 0.52$, $P = 0.48$, $R^2 = 0.02$), or head width ($F_{(1, 22)} = 0.15$, $P = 0.70$, $R^2 = 0.01$). Size of the home range was positively correlated with both the number of males ($F_{(1,23)} = 18.23$, $P = 0.0003$, $R^2 = 0.44$) and females ($F_{(1, 23)} = 6.48$, $P = 0.018$, $R^2 = 0.22$) overlapping his home range. Male SVL was not significantly related to the number of males ($F_{(1, 23)} = 0.17$, $P = 0.68$, $R^2 = 0.01$) or the number of females overlapping his home range ($F_{(1, 23)} = 0.03$, $P = 0.85$, $R^2 = 0.01$).

We obtained 482 observations for the 74 adult females identified from our site (all but one female was first sighted before winter). The minimum sightings required to determine female home ranges was 11, and therefore we were able to generate reliable home ranges for only 13 of the 74 females identified from the site. There was no significant difference ($F_{(1, 70)} = 0.01$, $P = 0.95$) in SVL between females with (64.54 ± 1.11 , $N = 13$) or without (64.46 ± 0.55 , $N = 61$) a generated

home range. The mean home range size for females was $188 \pm 17 \text{ m}^2$ ($100 - 300 \text{ m}^2$, $N = 13$); and this was not significantly different from that of the males ($F_{(1, 36)} = 0.18$, $P = 0.67$, log transformed). The mean number of males overlapping females was 8.5 (range of 4 – 13), while the mean intrasexual overlap for females was 9.7 (range of 4 – 15). The home range size of females was not significantly correlated with SVL ($F_{(1, 11)} = 0.62$, $P = 0.45$, $R^2 = 0.05$) or with the number of overlapped females ($F_{(1, 11)} = 1.57$, $P = 0.24$, $R^2 = 0.13$), but was positively correlated with the number of overlapped males ($F_{(1, 11)} = 8.57$, $P = 0.014$, $R^2 = 0.44$). As for males, female SVL was not significantly related to the number of males ($F_{(1, 11)} = 0.01$, $P = 0.98$, $R^2 = 0.01$) or to the number of females overlapping her home range ($F_{(1, 11)} = 0.36$, $P = 0.56$, $R^2 = 0.03$).

Number of copulation scars and female reproductive success

There was no relationship between the number of copulation scars (3.38 ± 0.18 , $N = 42$, range 1 – 6) and a female's SVL or her subsequent litter size ($N = 26$) ($P > 0.05$). In addition the number of recorded copulation scars had no significant effect on offspring mass, offspring SVL and offspring sprint speed (litter means) (all $P > 0.05$).

Litter size ranged from one to four (mean \pm SE = 1.8 ± 0.1). Maternal size (SVL) was significantly positively correlated with both litter size (Spearman rank correlation: $F_{(1, 41)} = 10.70$, $P = 0.002$, $R^2 = 0.21$) and litter mass ($F_{(1, 41)} = 13.72$, $P = 0.0006$, $R^2 = 0.25$), but there was no correlation with relative litter mass (litter mass/female mass; $P > 0.05$). Maternal size was also significantly positively correlated with mean offspring size (SVL: $F_{(1, 41)} = 4.83$, $P = 0.03$, $R^2 = 0.11$), but not with mean offspring mass or mean sprint speed of her offspring ($P > 0.05$).

Paternity and male reproductive success

We had 36 females from within the study area and nine females from the periphery of our study site, that may have mated with the known males from our home range study, for paternity analyses. The sire could be identified in 65% (52/80) of cases; 16 offspring did not have a father identified with no genetic mismatches, and the remaining 12 offspring had two potential fathers identified. Of the offspring born to females within the study area, 26% (10/38) were identified by overlap of the ranges of the parents (including all animals). Proximity distance (smallest distance between the closest edge of the home range of a female and all males) revealed that there were 15.3 males on average within 10 m of the female, and an additional 15.5 males on average within 20 m of the female. While 68% (21/31) of identified fathers were found within 20 m of the female, 32% (10/31) were found at greater distances from the female (Fig. 1).

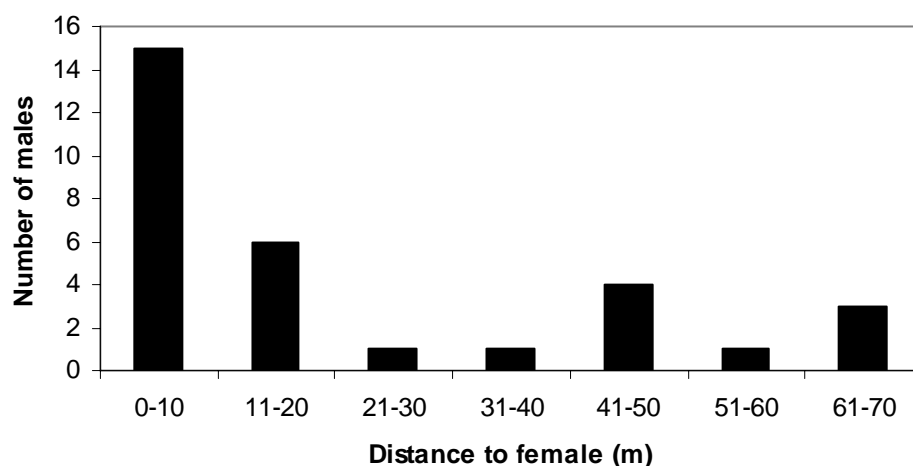


Figure 1 – Distances between the closest edge of the home ranges (MCP) of males and females (generated for all animals): specifically the distance between each mother and the identified fathers of their offspring.

Multiple paternity was evident in 94% (15/16) of litters in which more than one offspring had their sire identified: 87% (13/15) were sired by two males, and 14% (2/15) were sired by more than two males. Male reproductive success was variable (Fig. 2), with 28 males (of 54) identified as sires.



Figure 2 – Frequency distribution of male reproductive success in *N. ocellatus* as identified by microsatellites. Bars indicate how many males in the population fathered a particular number of offspring.

Sires (with one or more offspring identified) and nonsires did not differ significantly ($P > 0.05$) in any male characteristic measured (Table 1). Similarly, a stepwise multiple regression using eight male characteristics at the onset of the mating season (SVL, tail length, tail break length, head width, head length, mass, condition, number of females overlapped) found that these characteristics did not significantly predict male reproductive success (number of offspring sired) ($P > 0.05$). For those males for which home range size was generated, there was no correlation between home range size and reproductive success ($F_{(1, 11)} = 0.13$, $P = 0.73$).

Table 1 - Characteristics of adult sires and non sires in a population of *N. ocellatus*. SVL: snout-vent length, TL: tail length, TB: length to tail break, HW: head width, HL: head length, BM: body mass, BC: body condition(residuals), HR: home range size, FO: number of female home ranges overlapped.

	Sires		Nonsires	
	mean \pm SE	N	mean \pm SE	N
SVL (mm)	64.25 \pm 0.65	28	63.85 \pm 0.80	26
TL (mm)	68.85 \pm 2.54	27	71.27 \pm 3.21	26
TB (mm)	45.37 \pm 5.29	27	56.92 \pm 5.23	26
HW (mm)	8.52 \pm 0.09	27	8.38 \pm 0.10	25
HL (mm)	13.02 \pm 0.12	27	12.97 \pm 0.15	25
BM (mg)	4.22 \pm 0.15	27	4.05 \pm 0.15	24
BC	0.02 \pm 0.16	27	-0.03 \pm 0.24	24
HR (M ²)	208.7 \pm 27.7	13	224.7 \pm 31.6	12
FO	8.8 \pm 0.9	28	8.2 \pm 0.9	27

Discussion

Niveoscincus ocellatus exhibits high intra- and intersexual overlap of ranges between adults. Therefore, members of both sexes have multiple opportunities to encounter and mate with the opposite sex. Females of this species do mate multiple times, which is reflected by high levels of multiple paternity within litters; and some males are able to mate successfully with several different females.

There was no intersexual difference in calculated home range size for *N. ocellatus*, and the high intrasexual overlap between home ranges, especially between the males provides indirect evidence that *N. ocellatus* is a non-territorial species(Rose, 1981; Wone & Beauchamp, 2003). Similarly, there is no difference in

home range size between the sexes in other non-territorial skinks such as *Egernia major* (Osterwalder, Klingenbock & Shine, 2004) and *Tiliqua rugosa* (Bull & Freake, 1999). Furthermore, in our study, neither female nor male body size determined home range size [similar to other lizards, e.g., *Phrynosoma mcallii* (Wone & Beauchamp, 2003) and *Sceloporus virgatus* (Abell, 1999)], or the number of males or the number of females that overlapped. Therefore the only factor determining access to potential mates was home range size *per se*, and despite no clear intersexual differences in home range size, males had more access to females than females had to males, suggesting a non-random spatial pattern. As yet we do not know what predicts home range size of individuals of *N. ocellatus*.

Range overlap (including adults in which the minimum number of sightings were not recorded in order to determine an accurate home range) was not indicative of paternity: only 26% of sires were identified by range overlap of the parents. In some species, spatial proximity has been demonstrated to correspond better with male mating success than measures of home range overlap (Abell, 1997; Morrison, Keogh & Scott, 2002). However, due to the extended nature of our study as a result of two separate mating periods within the same breeding season (before and after winter) (Jones *et al.*, 1997), and the high numbers of males overlapping female home ranges, let alone in close proximity, spatial proximity is not very useful as a tool in determining likely paternity of a litter nor as an indicator of male reproductive success. Individuals of *N. ocellatus* tend to be quite cryptic. Mating has never been observed in the wild and, unlike other snow skinks (Olsson *et al.*, 2005), they do not mate guard. We expect that we missed many associations between the sexes, and, in conjunction with poor resolution of accurate home range sizes, we therefore view our observational data as a tool to inform our molecular results.

Niveoscincus ocellatus shows a high degree (94% of litters) of multiple paternity, one of the highest recorded in a lizard species, and an unexpected result given both the low litter sizes and low number of copulations suggested by the copulatory scars. Multiple paternity has similarly been recorded (albeit relatively recently) in other lizard species [75% of *N. microlepidotus* litters (Olsson *et al.*, 2005), 81% of *U. stansburiana* clutches (Zamudio & Sinervo, 2000), between 50.0% and 68.2 % of *L. vivipara* clutches (Laloi *et al.*, 2004), and 65% of *Eulamprus heatwolei* litters show multiple paternity (Morrison *et al.*, 2002)]. Thus, the widespread promiscuity of female lizards (Olsson & Madsen, 1998; Olsson & Madsen, 2001), seems to be associated with a very high level of multiple paternity in this taxon. Multiple paternity is also common in passerine birds [approximately 90% of passerine bird species have ‘extra-pair’ paternity (reviewed in Griffith, Owens & Thuman, 2002)], and among mammals (Stockley, 2003). The high level of multiple paternity in *N. ocellatus* confirms the notion that females are mating with multiple males (rather than multiple times with the same male) as suggested by the multiple copulatory scars on their torso (Jones *et al.*, 1997). With access to many males in close proximity, two temporally separate mating periods, and with the ability to store sperm over winter, females are providing a situation in which sperm competition occurs. The total number of mates (but not actual fathers) can be underestimated by genetic methods (Uller & Olsson, 2005), and may be more accurately determined by counting the mating scars, in particular in species such as *N. ocellatus* where they are distinct and permanent (but see Fitze *et al.*, 2005). As expected based on the low litter size, the mean number of copulation scars in *N. ocellatus* females was greater than the mean number of actual fathers but was not related to either female size or litter size. Furthermore, the number of scars did not influence offspring mass, size or

sprint speed. In contrast, larger female *L. vivipara* tend to be more promiscuous than smaller females (Fitze *et al.*, 2005), have smaller clutches and heavier offspring, and a lower incidence of infertile eggs (Uller & Olsson, 2005).

As is typical in non-monogamous systems, male reproductive success was variable in our study: 50% of successful males sired one offspring, 21% sired two and 25% sired more than two offspring. Similarly, Haenel *et al.* (2003) found that male reproductive success is variable in *Sceloporus undulatus*. Male size is commonly correlated with male mating success, for example in *Crotaphytus collaris* (Baird & Timanus, 1998) and *Ctenophorus ornatus* (Lebas, 2001). This occurs primarily in situations where males compete for territories and/or females. In our study, however, male fertilisation success was not correlated with any male phenotypic characteristic or home range size and/or number of females overlapped. The opportunity for male monopolisation of females is lower in non-territorial species and, consequently, variance in reproductive success and intrasexual selection on male traits may also be less pronounced. However, females from our population have small litter sizes resulting in low power to investigate male reproductive success. Females from a high elevation population have larger litter sizes (Wapstra & Swain, 2001a) and could provide a better system to investigate factors determining male reproductive success.

Overall, this species has a high degree of multiple paternity resulting from high access to the opposite sex and a high incidence of multiple mating. Multiple mating may result from a female strategy to ensure fertilisation, or from sexual conflict over mating decisions.

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References

- Abell AJ. 1997.** Estimating paternity with spatial behaviour and DNA fingerprinting in the striped plateau lizard, *Sceloporus virgatus* (Phrynosomatidae). *Behavioral Ecology & Sociobiology* **41**: 217-226.
- Abell AJ. 1999.** Male-female spacing patterns in the lizard, *Sceloporus virgatus*. *Amphibia-Reptilia* **20**: 185-194.
- Baird TA, Timanus DK. 1998.** Social inhibition of territorial behaviour in yearling male collared lizards, *Crotaphytus collaris*. *Animal Behaviour* **56**: 989-994.
- Birkhead TR, Moller AP. 1992.** *Sperm competition in birds: evolutionary causes and consequences*. Academic Press, London.

- Bull CM, Cooper SJB, Baghurst BC. 1998.** Social monogamy and extra-pair fertilization in an Australian lizard, *Tiliqua rugosa*. *Behavioral Ecology and Sociobiology* **44**: 63-72.
- Bull CM, Freake MJ. 1999.** Home-range fidelity in the Australian sleepy lizard, *Tiliqua rugosa*. *Australian Journal of Zoology* **47**: 125-132.
- Chapple DG, McCoull CJ, Swain R. 2002.** Changes in reproductive investment following caudal autonomy in viviparous skinks (*Niveoscincus metallicus*): lipid depletion or energetic diversion? *Journal of Herpetology* **36**: 480-486.
- Cooper WE Jr, Vitt LJ. 1993.** Female mate choice of large male broad-headed skinks. *Animal Behaviour* **45**: 683-693.
- Dodd CKJ. 1993.** The effects of toeclipping on sprint performance of the lizard *Cnemidophorus sexlineatus*. *Journal of Herpetology* **27**: 209-213.
- Eberhard WG. 1998.** Female roles in sperm competition. In: Birkhead TR and Moller AP, eds. *Sperm competition and sexual selection*. San Diego etc.: Academic Press.
- Fitze PS, Le Galliard JF, Federici P, Richard M, Clobert J. 2005.** Conflict over multiple-partner mating between males and females of the polygynandrous common lizards. *Evolution* **59**: 2451-2459.
- Gardner MG, Cooper SJB, Bull CM, Grant WN. 1999.** Isolation of microsatellite loci from a social lizard, *Egernia stokesii*, using a modified enrichment procedure. *The Journal of Heredity* **90**: 301-304.
- Griffith SC, Owens IPF, Thuman KA. 2002.** Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology* **11**: 2195-2212.

- Gullberg A, Olsson M, Tegelstrom H. 1997.** Male mating success, reproductive success and multiple paternity in a natural population of sand lizards: behavioural and molecular genetics data. *Molecular Ecology* **6**: 105-112.
- Haenel GJ, Smith LC, John-Alder HB. 2003.** Home-range analysis in *Sceloporus undulatus*. II. A test of spatial relationships and reproductive success. *Copeia*: 113-123.
- Hudson S. 1995.** Natural toe loss in southeastern Australian skinks: implications for marking lizards by toe-clipping. *Journal of Herpetology* **30**: 106-110.
- Hughes C. 1998.** Integrating molecular techniques with field methods in studies of social behaviour: a revolution results. *Ecology* **79**: 383-399.
- Jennions MD, Petrie M. 2000.** Why do females mate multiply? A review of the genetic benefits. *Biological Reviews* **75**: 21-64.
- Jones SM, Wapstra E, Swain R. 1997.** Asynchronous male and female gonadal cycles and plasma steroid concentrations in a viviparous lizard, *Niveoscincus ocellatus* (Scincidae), from Tasmania. *General and Comparative Endocrinology* **108**: 271-281.
- Kenward RE, Hodder KH. 1996.** *Ranges V. An analysis system for biological location data*. NERC, Dorset.
- Laloi D, Richard M, Lecomte J, Massot M, Clobert J. 2004.** Multiple paternity in clutches of common lizard *Lacerta vivipara*: data from microsatellite markers. *Molecular Ecology* **13**: 719-723.
- Lebas NR. 2001.** Microsatellite determination of male reproductive success in a natural population of the territorial ornate dragon lizard, *Ctenophorus ornatus*. *Molecular Ecology* **10**: 193-203.

- Lewis AR, Tirado G, Sepulveda J. 2000.** Body size and paternity in a teiid lizard (*Ameiva exsul*). *Journal of Herpetology* **34**: 110-120.
- Madsen T, Shine R, Loman J, Hakaansson T. 1992.** Why do female adders copulate so frequently? *Nature* **355**: 440-441.
- Marshall TC, Slate J, Kruuk LEB, Pemberton JM. 1998.** Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**: 639-655.
- M'Closkey RT, Deslippe RJ, Szpak CP. 1990.** Tree lizard distribution and mating system: the influence of habitat and food resources. *Canadian Journal of Zoology* **68**: 2083-2089.
- Melville J. 1998.** The evolution of locomotory mode in the lizard genus *Niveoscincus*: an ecomorphological analysis of ecology, behaviour, morphology and performance ability. Unpublished PhD thesis, University of Tasmania.
- Morrison SF, Keogh JS, Scott IAW. 2002.** Molecular determination of paternity in a natural population of the multiply mating polygynous lizard *Eulamprus heatwolei*. *Molecular Ecology* **11**: 535-545.
- O'Connor DE, Shine R. 2004.** Parental care protects against infanticide in the lizard *Egernia saxatilis* (Scincidae). *Animal Behaviour* **68**: 1361-1369.
- Olsson M, Gullberg A, Tegelstrom H. 1996a.** Mate guarding in male sand lizards (*Lacerta agilis*). *Behaviour* **133**: 367-386.
- Olsson M, Gullberg A, Tegelstrom H, Madsen T, Shine R. 1994.** Can female adders multiply? - Reply. *Nature* **369**: 528.

- Olsson M, Madsen T. 1998.** Sexual selection and sperm competition in reptiles. In: Birkhead TR and Moller AP, eds. *Sperm competition and sexual selection*. Cambridge: Academic press. 503-578.
- Olsson M, Madsen T. 2001.** Promiscuity in sand lizards (*Lacerta vivipara*) and adder snakes (*Vipera berus*): causes and consequences. *Journal of Heredity* **92**: 190-197.
- Olsson M, Shine R. 1996.** Does reproductive success increase with age or with size in species with indeterminate growth? A case study using sand lizards (*Lacerta agilis*). *Oecologia* **105**: 175-178.
- Olsson M, Shine R. 1997.** Advantages of multiple mating to female: a test of the infertility hypothesis using lizards. *Evolution* **51**: 1684-1688.
- Olsson M, Shine R, Madsen T, Gullberg A, Tegelstrom H. 1996b.** Sperm selection by females. *Nature* **383**: 585.
- Olsson M, Ujvari B, Wapstra E, Madsen T, Shine R, Bensch S. 2005.** Does mate guarding prevent rival mating in snow skinks? A test using AFLP. *Herpetologica* **61**: 389-394.
- Osterwalder K, Klingenbock A, Shine R. 2004.** Field studies on a social lizard: home range and social organization in an Australian skink, *Egernia major*. *Austral Ecology* **29**: 241-249.
- Ott JA, Scott DE. 1999.** Effects of toe-clipping and PIT-tagging on growth and survival in metamorphic *Ambystoma opacum*. *Journal of Herpetology* **33**: 344-348.
- Pearse DE, Janzen FJ, Avise JC. 2002.** Multiple paternity, sperm storage, and reproductive success of female and male painted turtles (*Chrysemys picta*) in nature. *Behavioral Ecology and Sociobiology* **51**: 164-171.

-
- Rawlinson PA. 1974.** Biogeography and ecology of the reptiles of Tasmania and the Bass Strait area. In: Williams WD, ed. *Biogeography and ecology in Tasmania*. The Hague: Junk, W. 291-388.
- Richard M, Lecomte J, de Fraipont M, Clobert J. 2005.** Age-specific mating strategies and reproductive senescence. *Molecular Ecology* **14**: 3147-3155.
- Rose B. 1981.** Factors affecting activity in *Sceloporus virgatus*. *Ecology* **62**: 706-716.
- Rose B. 1982.** Lizard home ranges. *Journal of Herpetology* **16**: 252-269.
- Ruby DE. 1984.** Male breeding success and differential access to females in *Anolis carolinensis*. *Herpetologica* **40**: 272-280.
- Smith RL. 1984.** *Sperm competition and the evolution of animal mating systems*. Academic Press, Orlando.
- Somma LA. 2003.** *Parental behavior in lepidosaurian and testudinian reptiles, a literature survey*. Krieger Publishing Company, Melbourne, Florida.
- Stockley P. 2003.** Female multiple mating behaviour, early reproductive failure and litter size variation in mammals. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**: 271-278.
- Stone PA, Baird TA. 2002.** Estimating lizard home range: the rose model revisited. *Journal of Herpetology* **36**: 427-436.
- Tregenza T, Wedell N. 2000.** Genetic compatibility, mate choice and patterns of parentage: invited review. *Molecular Ecology* **9**: 1013-1027.
- Uller T, Olsson M. 2005.** Multiple copulations in natural populations of lizards: evidence for the fertility assurance hypothesis. *Behaviour* **142**: 45-56.

- Wapstra E, Olsson M, Shine R, Edwards A, Swain R, Joss JMP. 2004.** Maternal basking behaviour determines offspring size in a viviparous reptile. *Proceedings Royal Society of London B (Suppl.)*: S230-S232.
- Wapstra E, O'Reilly J. 2001.** Potential 'costs of reproduction' in a skink: inter- and intrapopulation variation. *Austral Ecology* **26**: 179-186.
- Wapstra E, Swain R. 1996.** Feeding ecology of the Tasmanian spotted skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Australian Journal of Zoology* **44**: 205-213.
- Wapstra E, Swain R. 2001a.** Geographic and annual variation in life-history traits in a temperate zone Australian skink. *Journal of Herpetology* **35**: 194-203.
- Wapstra E, Swain R. 2001b.** Reproductive correlates of abdominal fat body mass in *Niveoscincus ocellatus*, a skink with an asynchronous reproductive cycle. *Journal of Herpetology* **35**: 403-409.
- Wapstra E, Swain R, Jones SM, O'Reilly J. 1999.** Geographic and annual variation in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Australian Journal of Zoology* **47**: 539-550.
- Wapstra E, Swain R, O'Reilly J. 2001.** Geographic variation in age and size at maturity in a small Australian viviparous skink. *Copeia* **2001**: 646-655.
- Wone B, Beauchamp B. 2003.** Movement, home range, and activity patterns of the horned lizard, *Phrynosoma mcallii*. *Journal of Herpetology* **37**: 679-686.
- Zamudio KR, Sinervo E. 2000.** Polygyny, mate-guarding, and posthumous fertilization as alternative male mating strategies. *Proceedings of the National Academy of Sciences of the United States of America* **97**: 14427-14432.

Chapter 6b

Paternal effects on offspring quality and survival in *Niveoscincus ocellatus*

Introduction

Parental care is rare in squamate (lizards, snakes and amphisbaenians) reptiles (exception e.g. O'Connor & Shine, 2004) and therefore paternal effects on offspring phenotype and fitness are largely restricted to nuclear genetic effects (Bernardo, 1996). There is a high occurrence of both multiple matings and multiple paternity in reptile species (e.g. Gullberg, Olsson & Tegelstrom, 1997; Laloi *et al.*, 2004; Olsson & Madsen, 1998; Pearse, Janzen & Avise, 2002; Uller & Olsson, 2005), providing the opportunity to investigate paternal effects without the confounding effect of different mothers. It is already known that the study species, *Niveoscincus ocellatus*, mates multiply (Jones, Wapstra & Swain, 1997; Wapstra *et al.*, 1999), and I have now demonstrated that this corresponds with a high degree (94%) of multiple paternity within litters (Chapter 6a). A role for genetic compatibility in mating outcomes (Lebas, 2002) is suggested by the evidence that females mated to multiple males have offspring with higher viability (Madsen *et al.*, 1992; Madsen *et al.*, 2005; Olsson *et al.*, 1994; reviewed in Tregenza & Wedell, 2000).

Using molecular techniques (microsatellites), I aim to identify the offspring that are sired by each of my study males (Chapter 6a), and, using this information, to determine the effect of paternal characteristics (in conjunction with maternal characteristics) on offspring characteristics at birth and their survivorship over

winter. Sire size within a litter has been shown to affect offspring size, condition and sex (Calsbeek & Sinervo, 2002). Genetic determinants of offspring dispersal have been shown to be significant in both *Uta stansburiana* (Sinervo *et al.*, 2006) and *Sceloporus occidentalis* (Massot *et al.*, 2003); natal dispersal is important for both inbreeding avoidance and competition for resources (Clobert *et al.*, 2001), which can have important consequences for offspring survival and fitness. Unfortunately, resultant sample sizes were too small to investigate the effect of parental characteristics on both offspring growth and dispersal. This was originally part of the study described in Chapter 6a. However, the section described here was omitted from the submitted manuscript (Chapter 6a) to provide that paper with a stronger focus for publishing purposes. It is included in the thesis because the combined chapters fall under the general theme of my thesis.

Methods

This study was conducted on a low elevation population of *N. ocellatus* near Orford (42°34'S, 147°52'E) on the east coast (50-75 m above sea level) of Tasmania. As part of the home range study in Chapter 6a, lizards were caught using mealworm “fishing”, noosing with long rods, or by hand. Every adult *N. ocellatus* observed in the study area was captured during February 2003 – December 2003, a time period encompassing both autumn and spring mating periods of the species. At first capture, all lizards were weighed (to the nearest 0.1 g) using a field balance; and snout vent length (SVL), tail length, distance from vent to tail break (to the nearest mm) and head length and head width in the males measured. A measure of body condition was obtained for the males by generating the residuals from a mass - SVL regression

($F_{(1,49)} = 76.15$, $P = 0.0001$, $R^2 = 0.61$). In males, the distal one cm portion of the tail was removed and stored in 90% ethanol for genotyping; tail autotomy and regeneration are common in snow skinks (Chapple, McCoull & Swain, 2002). By the end of the study, I had identified and marked 58 males and 74 females.

In late December 2003, all gravid females from my study site in the final stages of gestation (including any females caught on the periphery of my study site) were collected and returned to the laboratory for birth, as outlined in Chapter 6a. At birth, maternal length (SVL, tail length and length to tail break where applicable) and mass were recorded and a terminal tail sample (1 cm) was collected from the mother. A measure of maternal body condition after parturition was obtained by generating the residuals from a mass - SVL regression ($F_{(1,41)} = 45.32$ $P = 0.0001$, $R^2 = 0.53$). All offspring were measured (mass, SVL and tail length) and a measure of body condition was obtained by generating the Student residuals from a mass - SVL regression ($F_{(1,74)} = 117.54$, $P = 0.0001$, $R^2 = 0.61$). The offspring were toe-clipped for identification, a tail sample (0.5 cm) collected for genotyping, and sex was determined by hemipenes eversion (Harlow, 1996; Wapstra *et al.*, 2004). Sprint speed was measured (on the day of birth) using a computerised racetrack with five sets of photoelectric detectors: animals were warmed to 28 °C (Melville, 1998) for 30 min and were run once down a 2 m track, with the fastest speed (of four 50 cm sections within the track) being used for analysis as a measure of “best performance” (Wapstra & O'Reilly, 2001).

Survivorship of offspring

Offspring were held for up to five days in similar conditions to those for the gravid females; they were fed on alternate days with either nematodes (white worms)

or pear-banana baby food. The offspring were released at twelve release sites (randomly selected, with siblings released at different release sites) within their population of origin. I returned after winter (September/October 2004) to the study site and, during a four-week period, attempted to capture all juvenile lizards. Captured offspring were marked temporarily with a non-toxic paint pen to avoid a second recapture. When no unmarked offspring were observed at the field site or peripheral areas, I was confident that my recaptures represented a significant proportion of surviving offspring and that non-captures were likely to be non-survivors.

Paternity analysis

Paternity was assigned as described in Chapter 6a.

Statistical analyses

Data are presented as means \pm standard errors. All analyses were performed in SAS (v 6.12). Chi-squared analyses were used to analyse for differences in sex ratio and survivorship of offspring of the smallest and largest fathers within a litter (see Results), and multiple logistic regression (with forward selection) was used to determine effects on offspring survivorship. All other analyses were linear regressions or ANOVAs, unless stated. Where necessary, data were log-transformed to stabilise variances and/or normalise data: homogeneity of group variances was assessed by studying a plot of standard deviations against means, while assumptions of normality were checked by examining plots of standardized residuals against estimated values and the normal probability curve of the residuals.

Results

When paternal size effects were examined within litters in which multiple paternity was identified (the smallest versus the largest male the female successfully mated with; significant difference in size: ANOVA, $F_{(1,26)} = 23.34$, $P = 0.0001$), there was no significant effect of sire body size on offspring mass, SVL, tail length, condition and sprint speed ($P > 0.05$) (Table 1). In addition, there was no effect of sire body size on the sex ratio of their offspring or the subsequent offspring survival (Chi-squared analysis, $P > 0.05$).

Table 1 – Characteristics of the offspring of the smallest and the largest male siring offspring within each litter in *N. ocellatus*. SVL: snout-vent length, TL: tail length, BM: body mass, BC: body condition (residuals). Mean \pm SE (N).

	Smallest	Largest
SVL (mm)	29.31 \pm 0.23 (13)	29.35 \pm 0.16 (13)
TL (mm)	39.17 \pm 0.46 (13)	38.67 \pm 0.44 (13)
BM (mg)	524.10 \pm 11.52 (14)	544.89 \pm 13.50 (14)
BC	-0.26 \pm 0.31 (13)	0.30 \pm 0.27 (13)
Sprint speed (cm/s)	73.07 \pm 8.58 (10)	65.81 \pm 3.87 (11)
No. of sons	8	8
No. of daughters	5	7
No. of survivors	1/14	4/14

Survivorship of offspring

Offspring survival was 19% (15/80), and I identified the fathers for seven of these offspring. Seven fathers were represented by one surviving offspring, and 22

fathers were not represented by surviving offspring. A multiple logistic regression (forward selection) using seven characteristics of the father (SVL, tail length, tail break length, head width, head length, mass, body condition), five characteristics of the mother (SVL, tail length, tail break length, mass, body condition), and six characteristics of the offspring (SVL, mass, body condition, birth date, sprint speed, sex) found that there were no significant predictors of offspring survival, with no variables meeting the 0.05 significance level for entry into the model.

Discussion

Females of *N. ocellatus* do mate multiply, and I have shown that this corresponds with high levels of multiple paternity within litters and that some males are able to mate successfully with several different females (Chapter 6a). While I was unable to determine any significant predictors of male reproductive success in my population of *N. ocellatus*, within the 15 litters with multiple paternity I was able to investigate the consequences of females mating with smaller, versus larger, males. However, I found no measurable differences between the offspring of these two groups of males. Female size, in contrast, had effects on some offspring characteristics but not others (Chapter 6a). In contrast, work on *U. stansburiana* demonstrated that offspring sired by the larger male were larger and in better condition than offspring sired by the smaller male; in addition, offspring sired by the larger male were more likely to be sons, and offspring sired by the smaller male more likely to be daughters (Calsbeek & Sinervo, 2002). My study, however, revealed no effect of sire body size on the number of sons or daughters produced or survivorship of the offspring. Only female size is correlated with reproductive output (success) in this species (Chapter 6a), and

with offspring survival not correlated with either size or sex, there is little benefit for sex allocation in relation to the size of the father.

Furthermore, I was unable to demonstrate that any paternal, maternal and offspring characteristic measured, predicted offspring survivorship over winter. I have also previously demonstrated in this species that subjecting females to cold conditions at the end of gestation, which delays the timing of parturition, has no effect on the proportion of females represented by surviving offspring (Chapter 4, Atkins *et al.*, in press). Furthermore, while there was no treatment effect on dispersal of those offspring, there were varied negative effects on different aspects of growth as measured after release (Chapter 4, Atkins *et al.*, in press).

Overall, *N. ocellatus* has a high degree of multiple paternity resulting from both high access to the opposite sex and a high incidence of multiple mating. I can only speculate that the primary benefit females gain from mating multiply is to ensure fertilisation, with the identity of the father having little significant effect on either offspring characteristics at birth and on their subsequent survival over winter.

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References

- Atkins N, Swain R, Wapstra E, Jones SM. in press.** Late stage deferral of parturition in the viviparous lizard *Niveoscincus ocellatus* (Gray 1845): implications for offspring quality and survival. *Biological Journal of the Linnean Society*.
- Bernardo J. 1996.** Maternal effects in animal ecology. *American Zoologist* **36**: 83-105.
- Calsbeek R, Sinervo B. 2002.** Uncoupling direct and indirect components of female choice in the wild. *Proceedings of the National Academy of Science, USA* **99**: 14897-14902.
- Chapple DG, McCoull CJ, Swain R. 2002.** Changes in reproductive investment following caudal autotomy in viviparous skinks (*Niveoscincus metallicus*): lipid depletion or energetic diversion? *Journal of Herpetology* **36**: 480-486.
- Clobert J, Danchin E, Dhondt AA, Nichols JD. 2001.** *Dispersal*. Oxford University Press, Oxford.
- Gullberg A, Olsson M, Tegelstrom H. 1997.** Male mating success, reproductive success and multiple paternity in a natural population of sand lizards: behavioural and molecular genetics data. *Molecular Ecology* **6**: 105-112.
- Harlow PS. 1996.** A harmless technique for sexing hatchling lizards. *Herpetological Review* **27**: 71-72.
- Jones SM, Wapstra E, Swain R. 1997.** Asynchronous male and female gonadal cycles and plasma steroid concentrations in a viviparous lizard, *Niveoscincus ocellatus* (Scincidae), from Tasmania. *General and Comparative Endocrinology* **108**: 271-281.

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- Laloi D, Richard M, Lecomte J, Massot M, Clobert J. 2004.** Multiple paternity in clutches of common lizard *Lacerta vivipara*: data from microsatellite markers. *Molecular Ecology* **13**: 719-723.
- Lebas NR. 2002.** Mate choice, genetic incompatibility, and outbreeding in the ornate dragon lizard, *Ctenophorus ornatus*. *Evolution* **56**: 371-377.
- Madsen T, Shine R, Loman J, Hakaansson T. 1992.** Why do female adders copulate so frequently? *Nature* **355**: 440-441.
- Madsen T, Ujvari B, Olsson M, Shine R. 2005.** Paternal alleles enhance female reproductive success in tropical pythons. *Molecular Ecology* **14**: 1783-1787.
- Massot M, Huey RB, Tsuji JS, van Berkum FH. 2003.** Genetic, prenatal, and postnatal correlates of dispersal in hatchling fence lizards (*Sceloporus occidentalis*). *Behavioral Ecology* **14**: 650-655.
- Melville J. 1998.** The evolution of locomotory mode in the lizard genus *Niveoscincus*: an ecomorphological analysis of ecology, behaviour, morphology and performance ability. Unpublished D. Phil. Thesis, University of Tasmania.
- O'Connor DE, Shine R. 2004.** Parental care protects against infanticide in the lizard *Egernia saxatilis* (Scincidae). *Animal Behaviour* **68**: 1361-1369.
- Olsson M, Gullberg A, Tegelstrom H, Madsen T, Shine R. 1994.** Can female adders multiply? - Reply. *Nature* **369**: 528.
- Olsson M, Madsen T. 1998.** Sexual selection and sperm competition in reptiles. In: Birkhead TR and Moller AP, eds. *Sperm competition and sexual selection*. Cambridge: Academic press. 503-578.

- Pearse DE, Janzen FJ, Avise JC. 2002.** Multiple paternity, sperm storage, and reproductive success of female and male painted turtles (*Chrysemys picta*) in nature. *Behavioral Ecology and Sociobiology* **51**: 164-171.
- Sinervo B, Calsbeek R, Comendant T, Both C, Adamopoulou C, Clobert J. 2006.** Genetic and maternal determinants of effective dispersal: the effect of sire genotype and size at birth in side-blotched lizards. *American Naturalist* **168**: 88-99.
- Tregenza T, Wedell N. 2000.** Genetic compatibility, mate choice and patterns of parentage: invited review. *Molecular Ecology* **9**: 1013-1027.
- Uller T, Olsson M. 2005.** Multiple copulations in natural populations of lizards: evidence for the fertility assurance hypothesis. *Behaviour* **142**: 45-56.
- Wapstra E, Olsson M, Shine R, Edwards A, Swain R, Joss JMP. 2004.** Maternal basking behaviour determines offspring size in a viviparous reptile. *Proceedings Royal Society of London B (Suppl.)*: S230-S232.
- Wapstra E, O'Reilly J. 2001.** Potential 'costs of reproduction' in a skink: inter- and intrapopulational variation. *Austral Ecology* **26**: 179-186.
- Wapstra E, Swain R, Jones SM, O'Reilly J. 1999.** Geographic and annual variation in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Australian Journal of Zoology* **47**: 539-550.

Chapter 7

General Discussion

In the majority of viviparous squamates, pregnancy is essentially prolonged egg retention: there are simple placentae and embryonic nutrition is predominantly supplied through the yolk. In such cases, placentae are used primarily for the transport of gases, water and possibly some inorganic ions. However, significant placental transfer of nutrients (both organic and inorganic) may occur, and across this spectrum, transfer can either be facultative (supplemental) or obligate (Stewart, 1989). For those species in which placental nutrition has become more important, this is supported by a diverse range of placental arrangements (Blackburn, 2000; Blackburn, Vitt & Beuchat, 1984), and corresponds with a decreased reliance on nutrition supplied through the yolk.

The evolutionary transition from simple to complex placentation has occurred four times in lizards (Blackburn, 2000), and possibly once more in another reptilian lineage (Flemming & Blackburn, 2003; Flemming & Branch, 2001). With significant costs associated with producing and maintaining complex placentae, there must, therefore, be significant advantages to placentotrophy: these presumably relate to ecological situations in which lecithotrophy is limited (Jones & Swain, 2006). My thesis has explored the possible nature of these advantages using two species of viviparous lizards chosen for their different reproductive strategies and degrees of placentation. In particular, my thesis has addressed the “Maternal Manipulation” hypothesis (Shine, 1995; Webb, Shine & Christian, 2006), which suggests that females can enhance the fitness of their offspring by manipulating the embryonic environment.

My two study species, *Niveoscincus ocellatus* and *N. microlepidotus*, are both from a genus whose members have placentae of intermediate complexity, although there is considerable variation in placental structure and type between species (Stewart & Thompson, 1994; Stewart & Thompson, 1998; Thompson, Stewart & Speake, 2000; Weekes, 1930). In *N. ocellatus*, embryonic nutrition is known to be significantly placentotrophic (Thompson *et al.*, 2000), although *N. microlepidotus* had not been studied from this regard. I have now shown that in *N. microlepidotus* embryonic nutrition is predominantly lecithotrophic, although females may utilise facultative placentotrophy in some years (Chapter 3, Atkins, Swain & Jones, 2006b). Therefore, with both my model species able to provide at least some nutrients across the placenta, decisions concerning embryonic nutrition may be made not only several months prior to ovulation (i.e. during vitellogenesis) but also during embryonic development. Placental transfer can occur up until the time of parturition, with the transfer of lipids (^3H -oleic acid) demonstrated in late-stage embryos of *N. metallicus*, *N. ocellatus* and *N. microlepidotus* (Jones & Swain, 2006), and the transfer of amino acids (^3H -leucine) demonstrated in late stage embryos of *N. metallicus* (Swain & Jones, 1997).

Facultative placentotrophy may represent a significant selective advantage to viviparous reptiles with less complex placentae. I have demonstrated a capacity for facultative placentotrophy in *N. microlepidotus*, increasing the number of species in which this has been overtly documented [*Virginia striatula* (Stewart, 1989), *N. metallicus* (Thompson *et al.*, 1999a); *Pseudemoia spenceri* (Thompson *et al.*, 1999c) and *P. pagenstecheri* (Thompson *et al.*, 1999b)]. The ability of *N. microlepidotus* to utilise facultative placentotrophy allows mothers to supplement already adequate yolk supplies, which may enhance offspring condition (Swain & Jones, 2000a; Swain

& Jones, 2000b; Thompson *et al.*, 1999a; Thompson *et al.*, 1999b; Thompson *et al.*, 1999c). However, in *N. microlepidotus*, I was able to demonstrate this ability only in some years of my study: I suggest, therefore, that variation between years in food availability and/or environmental conditions in this species' alpine environment may preclude detectable facultative supplementation of yolk supplies in some, but not all, years.

In some viviparous squamates, only certain specific embryonic requirements (e.g. calcium in *Thamnophis ordinoides*, Stewart, Blackburn & Hoffman, 1990) are transferred by the placenta. In *N. microlepidotus*, placental transfer of lipid (³H-oleic acid) into embryos and embryonic abdominal fat bodies has been demonstrated (Jones & Swain, 2006). Future work should assess the transfer of other organic and inorganic nutrients, and quantify and compare the composition of eggs and neonates in *N. microlepidotus*. Such studies may provide evidence that at least some limited placentotrophy does occur every year, although levels of organic supplementation may be low.

My results, therefore, do not support the proposition that the capacity for facultative placentotrophy is a necessary condition for female viviparous lizards to be flexible with the timing of parturition (Jones & Swain, 2006), at least in *N. microlepidotus*. In the harsh natural environment experienced by this alpine species, females defer parturition from autumn until spring every year (Hutchinson, Robertson & Rawlinson, 1989; Olsson & Shine, 1998; Olsson & Shine, 1999). However, as discussed above, I could find no evidence for facultative placental transfer in some years of my study. I have demonstrated that in this species embryonic fat reserves built up during embryonic development in summer, decrease significantly over winter (Chapter 2, Atkins, Swain & Jones, in press), suggesting

that the embryos utilise these reserves through the winter. I suggest that these fat stores are derived from lecithotrophic energy stores: similarly, Rock (2005) suggested that components of metabolised yolk may be redistributed to the embryonic fat bodies for use over winter in the biennially reproducing gecko *Hoplodactylus maculatus*. I do not, however, discount the possibility that facultative placentotrophy may provide an additional advantage to offspring born in some years. In the only year of my study in which my results suggested a capacity for facultative placentotrophy in *N. microlepidotus*, I recorded the greatest spread of time over which births occurred, and this was the only year in which date of birth had no effect on any offspring characteristic measured (Chapter 3, Atkins *et al.*, 2006b). My study species, therefore, may represent a species which has not fully evolved the ability to utilise facultative placentotrophy to enable it to defer parturition over such a long period, over winter, and still relies on yolk nutrition to support its embryos. This may be a consequence of its harsh natural alpine environment preventing supplemental placental transfer in all years. Facultative placentotrophy may, however, allow females of my biennially reproducing species *N. microlepidotus* some flexibility in the exact timing of parturition in spring in response to proximate environmental conditions in some years.

My thesis investigated the major hypothesis that deferral of parturition after completion of embryonic development is a key strategy employed by females of viviparous lizards to maximise offspring fitness. The now considerable research into the selective pressures leading to the evolution of viviparity has led to the development of the “Maternal Manipulation” hypothesis, which suggests that females may enhance the fitness of their offspring by manipulating the thermal environment of their embryos via their own basking behaviour (Shine, 1995; Webb

et al., 2006). Diel distributions as well as mean incubation temperature are now known to be important for the developing embryo by significantly affecting developmental rates and/or offspring phenotypes, and therefore enhancing offspring fitness (Ji *et al.*, 2007; Shine, 2004; Webb *et al.*, 2006). It has been previously demonstrated that changes in offspring phenotype and performance can be generated by the basking opportunities of pregnant lizards (e.g. Schwarzkopf & Shine, 1991; Shine & Downes, 1999; Shine & Harlow, 1993). Previous work on snow skinks has also to some extent investigated the “Maternal Manipulation” hypothesis through research into the environmental manipulation of the maternal environment during gestation and its effect on offspring phenotype (Swain & Jones, 2000a; Swain & Jones, 2000b; Wapstra, 2000; Wapstra *et al.*, 2004). However, the environment into which the neonates are born also profoundly influences offspring fitness (Mathies & Andrews, 1995; Olsson & Shine, 1998; Rock, 2005; Swain & Jones, 2000a). My thesis suggests that another key form of “Maternal Manipulation” is the female’s ability to determine the time of parturition, which is one of the greatest determinants of offspring success (Bernardo, 1996). The timing of parturition in many mammalian species is influenced by environmental factors, for example in the Dall’s sheep, *Ovis dalli dalli*, in Alaska (Rachlow & Bowyer, 1994); red squirrels in Canada (Reale *et al.*, 2003) and in several bat species (Arlettaz *et al.*, 2001; Cumming & Bernard, 1997). I now propose that the “Maternal Manipulation” hypothesis should be extended to explain the ability of females of some viviparous squamate species to exhibit flexibility in the timing of parturition.

The results reported in the three interlinked chapters (Chapters 2, 3 and 4) in this thesis support my hypothesis that deferral of parturition is an important strategy for maximising offspring fitness in viviparous squamates. Enhanced

probability of survival has been suggested to be a benefit of delaying the birth of fully developed reptilian embryos by enabling further time for additional differentiation (Shine & Olsson, 2003). Deferral of parturition over winter in *N. microlepidotus*, which is clearly a successful strategy in this species, is not reliant on facultative placentotrophy. Although deferring parturition over winter clearly enhances offspring survival (Olsson & Shine, 1998), I have demonstrated that there are consequent trade-offs between offspring quality and offspring size (Chapter 2, Atkins *et al.*, in press). I compared spring-born neonates with autumn-born offspring delivered by dissection. Although offspring born in spring exhibited lower growth rates, reduced sprint speed after birth, reduced condition and decreased energy reserves compared with autumn-born young, they are both heavier and longer than autumn-born neonates. In reptiles, larger neonatal size is often an indicator of offspring quality (Bernardo, 1996) and a strong indicator of the probability of survival (e.g. Civantos, Salvador & Veiga, 1999; Ferguson & Fox, 1984; Fox, 1978; Sinervo & Doughty, 1996). There may be other benefits to delaying parturition: in *N. microlepidotus*, despite effects such as reduced growth rates (as measured in the laboratory) and decreased energy stores, spring-born offspring that do survive may expect many months of equable weather to grow and accumulate further energy reserves before entering hibernation the following winter. Thus, my results, in addition to previous work by Olsson & Shine (1998), provide strong evidence to support the “Maternal Manipulation” hypothesis, in that timing of parturition on this longer time scale is also an important maternal manipulation.

It will be important to test these ideas on other lineages and in species with different placental types. The placental complexity and degree of placentotrophy of *Pseudemoia entrecasteauxii* are similar to those of *N. ocellatus*, while *P.*

pagenstecheri has significantly greater dependence on placentotrophy (Stewart & Thompson, 1993; Stewart & Thompson, 1996; Thompson & Stewart, 1994; Thompson *et al.*, 2000; Thompson *et al.*, 1999b). Assessment of the ability of these species to defer parturition in response to unsuitable environmental conditions, and their capacity for facultative placentotrophy would help to elucidate whether a capacity for facultative placentotrophy provides an important selective advantage to viviparous reptiles with less complex placentae.

I also investigated the effect of date of birth in spring on offspring characteristics in *N. microlepidotus*. While results varied between years (Chapter 3, Atkins *et al.*, 2006b), my results show that in some years it is advantageous for females to delay the birth of their offspring: there is no reduction in offspring condition or embryonic fat reserves and resulting offspring are either larger or with enhanced growth rates than those born earlier in spring. This observed variation between years may be attributed to the harsh natural environment experienced by this species, affecting maternal provisioning through both the yolk and the placenta (Chapter 3, Atkins *et al.*, 2006b; Swain & Jones, 2000a) and the environment into which the neonates are to be born. Shine & Olsson (2003) have previously demonstrated that offspring survival in *N. microlepidotus* was enhanced by a later parturition date in spring. My work, in which I investigated the effect on a larger range of offspring characteristics, enlarges upon theirs. I have demonstrated that offspring fitness may be significantly affected by birth date in this species, thus providing support for the hypothesis that smaller scale variation in the natural timing of parturition represents another important maternal effect in the biennially reproducing *N. microlepidotus*.

However, when females from both of my study species were experimentally induced to defer parturition [4 weeks for *N. microlepidotus* females (Chapter 2, Atkins *et al.*, in press) and up to 3 weeks for *N. ocellatus* females (Chapter 4, Atkins *et al.*, 2007)], at the time of natural parturition, there was a less marked effect on offspring characteristics. The ability of these females to defer parturition in response to adverse environmental conditions provides further support that small scale variation, in addition to large scale variation, in the timing of parturition is also an important maternal manipulation, providing evidence to support the “Maternal Manipulation” hypothesis.

In comparison to *N. microlepidotus*, *N. ocellatus* has a reduced ability to defer parturition at the time of natural parturition, at least in the high elevation population studied. Both species differ markedly in the timing of key reproductive events, namely the timing of birth relative to the onset of winter: with births occurring in spring in *N. microlepidotus* (Hutchinson *et al.*, 1989; Olsson & Shine, 1998; Olsson & Shine, 1999) and early to late summer in *N. ocellatus* (Wapstra *et al.*, 1999). The reduced advantage of deferring parturition due to the timing of winter, in conjunction with the greater reliance on obligate placentotrophy (Chapter 1), I suggest explain the difference in abilities of females from the different species to defer parturition.

Variation in the timing of parturition is an important maternal manipulation, however, my research revealed that the ability to defer parturition also differed between populations of *N. ocellatus* (Chapter 4, Atkins *et al.*, 2007). In conjunction with marked geographic differences in the reproductive cycle of this species (Wapstra *et al.*, 1999), there are also other significant differences such as age and size at maturity between populations (Wapstra & Swain, 2001; Wapstra, Swain &

O'Reilly, 2001). Furthermore, in the high elevation population, both the constraints of the shorter breeding season and reduced time between births and the onset of winter in relation to low elevation populations (Wapstra *et al.*, 1999) reduce the advantages of being able to defer birth in response to proximate environmental variation. Previous research on *N. metallicus* has showed that the degree of placentotrophy (measured by comparing the dry weights of eggs and neonates) can differ considerably between populations of the same species (Thompson *et al.*, 1999a; Thompson *et al.*, 2000). The possibility that differences in the abilities of female *N. ocellatus* from different populations to defer parturition may also reflect differences in the degree of placentotrophy between those populations warrants further research. A difference in the degree of placentotrophy may reflect a difference in the structure of the placentae in each population [not elucidated for the two populations of *N. metallicus* (Thompson *et al.*, 2000)] and/or difference in the amount of facultative transfer that can occur due to environmental constraints.

This study has revealed that two closely related species (*N. microlepidotus* and *N. ocellatus*) of viviparous lizard differ in both the length of time for which females are able to defer parturition and in their ability to hold fully developed embryos successfully: however, the physiological mechanisms behind this ability have not been fully determined. To test how deferral of parturition may occur in response to unsuitable environment conditions, I investigated the potential for environmental modulation of either the endocrine cascade initiating parturition or of oviductal innervation (Chapter 5, Atkins, Jones & Guillette Jr., 2006a).

The most important proximate environmental factor controlling the timing of reproduction in reptilian species is temperature (Licht, 1984), and therefore I investigated the role of temperature as a proximate factor controlling the timing of

parturition. Guillette *et al.* (1991) hypothesised that parturition in viviparous reptiles is potentiated by the removal of a β -adrenergic block on oviductal contractions. *Niveoscincus microlepidotus* provided me with an ideal model to investigate this hypothesis because completion of embryonic development, which is normally associated with the initiation of parturition, is separated from the natural time of parturition by 5-6 months (Hutchinson *et al.*, 1989; Olsson & Shine, 1998; Olsson & Shine, 1999); furthermore, females are able to defer parturition in the spring for an additional 4 weeks when subjected to adverse environmental conditions (Chapter 2, Atkins *et al.*, in press). My hypothesis that uterine preparations of *N. microlepidotus* would be responsive to exogenous hormones (AVT and PGF_{2 α}) in both autumn and spring was supported. This clearly demonstrates that lack of uterine responsiveness is not the factor preventing parturition. My results suggest that in this species and also in *N. ocellatus*, that β -adrenergic stimulation probably mediates environmental factors, and allows females to defer parturition. Indeed, I have demonstrated that environmental (temperature) modulation of either the endocrine cascade initiating parturition or of oviductal innervation influences the timing of parturition: subjecting females to cold conditions towards the end of gestation can prevent the initiation of parturition. These results highlight the key role of temperature as an important proximate factor controlling the timing of parturition in viviparous squamates. However, temperature is unlikely to be the only factor: further research is required to investigate the potential roles of photoperiod and seasonal hormone patterns in the control of the timing of parturition (Girling, Jones & Swain, 2002).

However, it is also important to consider other factors influencing offspring fitness besides the timing of parturition: the mating system is of fundamental importance to offspring fitness. In Chapter 6, I considered explicitly the links

between the mating system and offspring phenotype and fitness in *N. ocellatus*. In my study population, I was able to show that individual females had access to many males. There was a corresponding high level of multiple paternity (93%) within litters, and some males mated successfully with several different females (Chapter 6a). A high incidence of multiple paternity has been reported in several other lizard species: e.g. *N. microlepidotus* (Olsson *et al.*, 2005), *Uta stansburiana* (Zamudio & Sinervo, 2000) and *Eulamprus heatwolei* (Morrison, Keogh & Scott, 2002). A pattern is beginning to emerge that suggests that squamates are similar to other taxa in displaying high levels of multiple paternity; for example in passerine birds [approximately 90% of passerine bird species have 'extra-pair' paternity (reviewed in Griffith, Owens & Thuman, 2002)] and mammals (Stockley, 2003). I was unable to relate either the male characteristics measured or home range attributes of males to male reproductive success in this population. This result was not wholly unexpected given the extended length of the mating season and large number of potential males. Strategies, such as mate guarding and female defence via defined territories (Olsson & Madsen, 1998; Tregenza & Wedell, 2000), which occur in many other squamates, are unlikely to be effective. I cannot rule out other male behaviours and characters contributing to success: for example, a strong link between male whole body performance (sprint speed) and reproductive success has been established in other squamates (e.g. Husak *et al.*, 2006). Such factors could certainly apply in my species, especially as I recorded significant distances between females and the sire/s of their offspring. I was also unable to find any effect of male characteristics on offspring characteristics at birth (including sex) or offspring survival over winter (Chapter 6b). These results contrast with other studies: for example in *Uta stansburiana*, male size within a litter is correlated with several offspring characteristics (Calsbeek &

Sinervo, 2002). I speculate that in *N. ocellatus* the only benefit females gain from mating multiply is to ensure fertilisation (Olsson & Shine, 1997; Uller & Olsson, 2005).

This thesis has demonstrated that females of the viviparous lizards *N. microlepidotus* and *N. ocellatus* exhibit a suite of characteristics not available to oviparous species, that enable them to manipulate offspring characteristics through controlling the timing of parturition. This has provided new evidence to support Shine's "Maternal Manipulation" hypothesis (Shine, 1995; Webb *et al.*, 2006) and has further highlighted the significance of viviparity as a maternal effect in viviparous reptiles.

References

- Arlettaz R, Christe P, Lugon A, Perrin N, Vogel P. 2001.** Food availability dictates the timing of parturition in insectivorous mouse-eared bats. *Oikos* **95**: 105-111.
- Atkins N, Jones SM, Guillette Jr. LJ. 2006a.** Timing of parturition in two species of viviparous lizard: influences of beta-adrenergic stimulation and temperature upon uterine responses to arginine vasotocin (AVT). *Journal of Comparative Physiology B* **176**: 783-792.
- Atkins N, Swain R, Jones SM. 2006b.** Does date of birth or a capacity for facultative placentotrophy influence offspring quality in a viviparous skink, *Niveoscincus microlepidotus*? *Australian Journal of Zoology* **54**: 369-374.

-
- Atkins N, Swain R, Jones SM. in press.** Are babies better in autumn or spring? The consequences of extending gestation in a biennially reproducing viviparous lizard. *Journal of Experimental Zoology*.
- Atkins N, Swain R, Wapstra E, Jones SM. 2007.** Late stage deferral of parturition in the viviparous lizard *Niveoscincus ocellatus* (Gray 1845): implications for offspring quality and survival. *Biological Journal of the Linnean Society* **90**: 735-746.
- Bernardo J. 1996.** Maternal effects in animal ecology. *American Zoologist* **36**: 83-105.
- Blackburn DG. 2000.** Reptilian viviparity: past research, future directions, and appropriate models. *Comparative Biochemistry and Physiology Part A* **127**: 391-409.
- Blackburn DG, Vitt LJ, Beuchat CA. 1984.** Eutherian-like reproductive specialisations in a viviparous reptile. *Proceedings of the National Academy of Science, USA* **81**: 4860-4863.
- Calsbeek R, Sinervo B. 2002.** Uncoupling direct and indirect components of female choice in the wild. *Proceedings of the National Academy of Science, USA* **99**: 14897-14902.
- Civantos E, Salvador A, Veiga JP. 1999.** Body size and microhabitat affect winter survival of hatchling *Psammodromus algirus* lizards. *Copeia* **1999**: 1112-1117.
- Cumming GS, Bernard RTF. 1997.** Rainfall, food abundance and timing of parturition in African bats. *Oecologia* **111**: 309-317.

- Ferguson GW, Fox SF. 1984.** Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. *Evolution* **38**: 342-349.
- Flemming AF, Blackburn DG. 2003.** Evolution of placental specializations in viviparous African and South American lizards. *Journal of Experimental Zoology Part a-Comparative Experimental Biology* **299A**: 33-47.
- Flemming AF, Branch WR. 2001.** Extraordinary case of matrotrophy in the African skink *Eumecia anchietae*. *Journal of Morphology* **247**: 264-287.
- Fox SF. 1978.** Natural selection on behavioural phenotypes of the lizard *Uta stansburiana*. *Ecology* **59**: 834-847.
- Girling JE, Jones SM, Swain R. 2002.** Induction of parturition in snow skinks: can low temperatures inhibit the actions of AVT? *Journal of Experimental Zoology* **293**: 525-531.
- Griffith SC, Owens IPF, Thuman KA. 2002.** Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology* **11**: 2195-2212.
- Guillette Jr. LJ, Dubois DH, Cree A. 1991.** Prostaglandins, oviductal function, and parturient behaviour in nonmammalian vertebrates. *American Journal of Physiology* **260**: R854-R861.
- Husak JF, Fox SF, Lovern MB, Van Den Bussche RA. 2006.** Faster lizards sire more offspring: sexual selection on whole-animal performance. *Evolution* **60**: 2122-2130.
- Hutchinson MN, Robertson P, Rawlinson PA. 1989.** Redescription and ecology of the two endemic Tasmanian scincid lizards *Leiopisma microlepidotum* and

L. pretiosum. *Papers and Proceedings of the Royal Society of Tasmania* **123**: 257-274.

Ji X, Lin C-X, Lin L-H, Qui Q-B, Du Y. 2007. Evolution of viviparity in warm-climate lizards: an experimental test of the maternal manipulation hypothesis. *Journal of Evolutionary Biology* **20**: 1037-1045.

Jones SM, Swain R. 2006. Placental transfer of H-3-oleic acid in three species of viviparous lizards: a route for supplementation of embryonic fat bodies? *Herpetological Monographs*: 186-193.

Licht P. 1984. Reptiles. In: Lamming GE, ed. *Marshall's physiology of reproduction*. 4th ed. Edinburgh: Churchill Livingstone. 206-282.

Mathies T, Andrews RM. 1995. Thermal and reproductive biology of high and low elevation populations of the lizard *Sceloporus scalaris* - implications for the evolution of viviparity. *Oecologia* **104**: 101-111.

Morrison SF, Keogh JS, Scott IAW. 2002. Molecular determination of paternity in a natural population of the multiply mating polygynous lizard *Eulamprus heatwolei*. *Molecular Ecology* **11**: 535-545.

Olsson M, Madsen T. 1998. Sexual selection and sperm competition in reptiles. In: Birkhead TR and Moller AP, eds. *Sperm competition and sexual selection*. Cambridge: Academic press. 503-578.

Olsson M, Shine R. 1997. Advantages of multiple mating to female: a test of the infertility hypothesis using lizards. *Evolution* **51**: 1684-1688.

Olsson M, Shine R. 1998. Timing of parturition as a maternal care tactic in an alpine lizard species. *Evolution* **52**: 1861-1864.

Olsson M, Shine R. 1999. Plasticity of frequency of reproduction in an alpine lizard, *Niveoscincus microlepidotus*. *Copeia* **1999**: 794-796.

-
- Olsson M, Ujvari B, Wapstra E, Madsen T, Shine R, Bensch S. 2005.** Does mate guarding prevent rival mating in snow skinks? A test using AFLP. *Herpetologica* **61**: 389-394.
- Rachlow JL, Bowyer RT. 1994.** Variability in maternal-behavior by Dalls Sheep - environmental tracking or adaptive strategy. *Journal of Mammalogy* **75**: 328-337.
- Reale D, McAdam AG, Boutin S, Berteaux D. 2003.** Genetic and plastic responses of a northern mammal to climate change. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**: 591-596.
- Rock J. 2005.** Delayed parturition: constraint or coping mechanism in a viviparous gekkonid? *Journal of Zoology (London)* **268**: 355-360.
- Schwarzkopf L, Shine R. 1991.** Thermal biology of reproduction in viviparous skinks, *Eulamprus tympanum* - why do gravid females bask more. *Oecologia* **88**: 562-569.
- Shine R. 1995.** A new hypothesis for the evolution of viviparity in reptiles. *The American Naturalist* **145**: 809-823.
- Shine R. 2004.** Does viviparity evolve in cold climate reptiles because pregnant females maintain stable (not high) body temperatures? *Evolution* **58**: 1809-1818.
- Shine R, Downes SJ. 1999.** Can pregnant lizards adjust their offspring phenotypes to environmental conditions? *Oecologia* **119**: 1-8.
- Shine R, Harlow P. 1993.** Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia* **96**: 122-127.

-
- Shine R, Olsson M. 2003.** When to be born? Prolonged pregnancy or incubation enhances locomotor performance in neonatal lizards (Scincidae). *Journal of Evolutionary Biology* **16**: 823-832.
- Sinervo B, Doughty P. 1996.** Interactive effects of offspring size and timing of reproduction on offspring reproduction: experimental, maternal, and quantitative genetic aspects. *Evolution* **50**: 1314-1327.
- Stewart JR. 1989.** Facultative placentotrophy and the evolution of squamate placentation: quality of eggs and neonates in *Virginia striatula*. *The American Naturalist* **133**: 111-137.
- Stewart JR, Blackburn DG, Hoffman LH. 1990.** Nutritional provision to embryos in a predominantly lecithotrophic placental reptile, *Thamnophis ordinoides* (Squamata: Serpentes). *Physiological Zoology* **63**: 722-734.
- Stewart JR, Thompson MB. 1993.** A novel pattern of embryonic nutrition in a viviparous reptile. *Journal of Experimental Biology* **174**: 97-108.
- Stewart JR, Thompson MB. 1994.** Placental structure of the Australian lizard, *Niveoscincus metallicus* (Squamata: Scincidae). *Journal of Morphology* **220**: 223-236.
- Stewart JR, Thompson MB. 1996.** Evolution of reptilian placentation: development of extraembryonic membranes of the Australian scincid lizards, *Bassiana duperreyi* (oviparous) and *Pseudomoia entrecasteauxii* (viviparous). *Journal of Morphology* **227**: 349-370.
- Stewart JR, Thompson MB. 1998.** Placental ontogeny of the Australian scincid lizards *Niveoscincus coventryi* and *Pseudomoia spenceri*. *Journal of Experimental Zoology* **282**: 535-559.

- Stockley P. 2003.** Female multiple mating behaviour, early reproductive failure and litter size variation in mammals. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**: 271-278.
- Swain R, Jones SM. 1997.** Maternal transfer of 3H-labelled leucine in the viviparous lizard *Niveoscincus metallicus* (Scincidae: Lygosominae). *Journal of Experimental Zoology* **277**: 139-145.
- Swain R, Jones SM. 2000a.** Facultative placentotrophy: half-way house or strategic solution? *Comparative Biochemistry and Physiology Part A* **127**: 441-451.
- Swain R, Jones SM. 2000b.** Maternal effects associated with gestation conditions in a viviparous lizard, *Niveoscincus metallicus*. *Herpetological Monographs* **14**: 432-440.
- Thompson MB, Speake BK, Stewart JR, Russell K, McCartney RJ, Surai PF. 1999a.** Placental nutrition in the viviparous lizard *Niveoscincus metallicus*: the influence of placental type. *Journal of Experimental Biology* **202**: 2985-2997.
- Thompson MB, Stewart JR. 1994.** Egg and clutch size of the viviparous Australian skink *Pseudemoia pagenstecheri* and the identity of species with type III allantoplacentae. *Journal of Herpetology* **28**: 519-521.
- Thompson MB, Stewart JR, Speake BK. 2000.** Comparison of nutrient transport across the placenta of lizards differing in placental complexity. *Comparative Biochemistry and Physiology Part A* **127**: 469-479.
- Thompson MB, Stewart JR, Speake BK, Russell K, McCartney RJ, Surai PF. 1999b.** Placental nutrition in a viviparous lizard (*Pseudemoia pagenstecheri*) with a complex placenta. *Journal of Zoology, (London)* **248**: 295-305.

Thompson MB, Stewart JR, Speake BK, Russell KJ, McCartney RJ. 1999c.

Placental transfer of nutrients during gestation in the viviparous lizard,
Pseudemoia spenceri. *Journal of Comparative Physiology B* **169**: 319-328.

Tregenza T, Wedell N. 2000. Genetic compatibility, mate choice and patterns of
parentage: invited review. *Molecular Ecology* **9**: 1013-1027.

Uller T, Olsson M. 2005. Multiple copulations in natural populations of lizards:
evidence for the fertility assurance hypothesis. *Behaviour* **142**: 45-56.

Wapstra E. 2000. Maternal basking opportunity affects juvenile phenotype in a
viviparous lizard. *Functional Ecology* **14**: 345-353.

Wapstra E, Olsson M, Shine R, Edwards A, Swain R, Joss JMP. 2004. Maternal
basking behaviour determines offspring size in a viviparous reptile.
Proceedings Royal Society of London B (Suppl.): S230-S232.

Wapstra E, Swain R. 2001. Geographic and annual variation in life-history traits in
a temperate zone Australian skink. *Journal of Herpetology* **35**: 194-203.

Wapstra E, Swain R, Jones SM, O'Reilly J. 1999. Geographic and annual variation
in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus*
ocellatus (Squamata: Scincidae). *Australian Journal of Zoology* **47**: 539-550.

Wapstra E, Swain R, O'Reilly J. 2001. Geographic variation in age and size at
maturity in a small Australian viviparous skink. *Copeia* **2001**: 646-655.

Webb JK, Shine R, Christian KA. 2006. The adaptive significance of reptilian
viviparity in the tropics: testing the maternal manipulation hypothesis.
Evolution **60**: 115-122.

Weekes HC. 1930. On placentation in reptiles. *Proceedings of the Linnean Society*
of New South Wales **55**: 550-576.

Zamudio KR, Sinervo E. 2000. Polygyny, mate-guarding, and posthumous fertilization as alternative male mating strategies. *Proceedings of the National Academy of Sciences of the United States of America* **97**: 14427-14432.

Appendices

Appendix 1

Successful treatment of a mite infestation in gravid spotted snow skinks (*Niveoscincus ocellatus*)

Manuscript accepted as: Atkins NM, Wapstra E. 2004. Successful treatment of a mite infestation in gravid spotted snow skinks (*Niveoscincus ocellatus*). *Herpetofauna* 34 (2): 66-69.

Introduction

Over 250 species of mites have been identified as parasites of reptiles (Mader, 1996; Mader, 1989 and references therein). The introduced snake mite, *Ophionyssus natricis*, is the species usually associated with most captive population problems (Weigal, 1988). While many individuals may carry low numbers of mites, potentially life threatening anaemia can result from the mites' ability to ingest large quantities of blood from their host (Mader, 1989). The transition from healthy reptiles in captivity to reptiles with major problems associated with excessive mites can be extremely rapid, with death from anaemia potentially occurring within days. In addition, the mites may also transmit blood parasites with detrimental effects on the host (Oppliger *et al.*, 1996). Less obvious and directly observable effects (although still highly undesirable from a research and/or breeding viewpoint) include effects of maternal parasites on offspring phenotypic characters such as body mass, performance characteristics, dispersal behaviour and survival (Sorci *et al.*, 1994; Sorci & Clobert, 1995; Sorci *et al.*, 1996).

While mites occur naturally on individuals in wild populations, it is often not until reptiles are confined to a limited space in captivity that their numbers and density increase dramatically - potentially leading to major problems. Commonly - though not confined to - mite infestations are associated with unsanitary cage conditions and poor husbandry practices. First signs of infestation may be that of depression, dull or lacklustre appearance, lack of appetite or obvious disease in the affected host (Mader, 1989). Early detection can be made simply by examining the animal's scales: inflammation caused by the mites will usually push the scales up into a somewhat raised position (Weigel, 1988). Mites will also accumulate around areas such as the eyes, ears and vent, and in extreme cases, the animal will have mites visibly crawling around its body. Mites will also disperse within the cage, with egg development occurring in the substrate (Mader, 1989).

There is no single treatment that is both 100% effective and safe for the eradication of mites on reptiles in captivity (Mader, 1996). Several methods that have been reported in the literature recently include: immersing the reptiles in vegetable oil (Espinoza *et al.*, 1998); capture of mobile mites by masking tape (Mahaney, 1995); and removal by hand of gravid females and egg cases, with simplification of the cage environment to reduce the number of potential ovipositional sites (Montanucci, 1997). However, when large numbers of animals are maintained, as is often the case for research and/or breeding purposes, these methods may be labour intensive and not overly effective. To prevent outbreaks in a captive population, it can be useful to treat new arrivals routinely, whether or not signs of infestation are detected (Weigel, 1988).

Mite problems, identification and treatment

At the University of Tasmania, Australia, research is conducted on a variety of skink species, particularly snow skinks (*Niveoscincus*), examining reproductive tactics and life history evolution. This necessitates the maintenance of large numbers of skinks annually (up to 300 gravid females) during their gestation (up to 4 months). Snow skinks routinely carry "pocket mites" (species unknown) that appear in the skin folds around the forelegs in numbers up to 50+. These mites, however, do not appear to proliferate in laboratory conditions and appear to cause few problems (Blomberg, 1995). However, in addition, snow skinks also carry another species of mite that is capable of causing major problems.

As part of the research routine, all animals are usually treated (treatment described in full below) upon arrival at the laboratory with *Neguvon*® (Bayer, Aus., Ltd.), an anticholinesterase compound in soluble powder form, commonly used for bot (fly larvae and eggs) control in horses and worm control in cattle. However, on one occasion (season 2002/2003) during an ongoing study on *Niveoscincus ocellatus* health problems were observed. *Niveoscincus ocellatus* are a small (up to 10 g, 70 mm snout-vent length) annually breeding viviparous skink common in lowland and highland rock screes throughout Tasmania (Wapstra *et al.*, 1999). Gravid females were collected in mid-late November, and treated once with *Neguvon*® upon arrival at the laboratory. Parturition commenced as expected in late December, although all births were stillbirths. Upon close inspection, the animals were found to be heavily mite-infested, with the majority of scales on the tail lifted, and evidence of anaemia shown by pale tongues (normally dark red/blue) and pale gums. On reflection, a noticeable decrease in basking of the gravid females later in gestation could have

been a warning sign, although feeding did not appear to be affected. All females (including the remaining gravid individuals) were immediately re-treated, with a follow-up 2 weeks later, with *Neguvon*® (as described below). In addition, all cages and cage accessories were cleaned thoroughly in hot soapy water, rinsed and left to dry and later sprayed with a *Neguvon*® solution (same concentration as for dipping) and the shelves in the laboratory cleaned and sprayed with both a *Neguvon*® solution and *Baygon*® surface insecticide spray (Active ingredients: 10 g/kg Propoxur, 0.4 g/kg Cyfluthrin, 0.4 g/kg Transfluthrin). Mites can certainly move between suitable habitats (i.e., cages) and the surface spray was effective in preventing this, as evidenced by dead mites on the surface of the shelves.

These two treatments were effective in controlling the outbreak, illustrating that the outbreak was a result of not re-dipping the captive population after an initial treatment, not failure of the product to work. After re-treatment following the mite outbreak, there was approximately 10 days between the last stillbirth and the first healthy birth, with all subsequent births resulting in healthy neonates. After treatment, females were provided with water *ad libitum* and fed mealworms dusted with multivitamin and mineral mix and additionally had their diet supplemented with mashed beef liver to treat the anaemia. Of the 80 females treated, all survived except for one that was later realized to have been shedding at the time of treatment. The mite was identified as *Ophionyssus scincorum* (Domrow *et al.*, 1980; Domrow, 1985), a mite associated with skinks in Australasia.

Treatment regime, application and dosage

Concern has been raised about products such as *Neguvon*® for control of mite infestations in reptiles (Mahaney, 1995; Montanucci, 1997; Espinoza *et al.*, 1998), particularly for gravid individuals. However, when used carefully, *Neguvon*® is a highly effective and relatively safe remedy for mite infestations in reptiles (Weigel, 1988) and, as illustrated above, poor control can lead to more significant problems. Our laboratory policy is that all lizards are treated for mites when they first enter the laboratory and again 14 days after first treatment. Snow skinks, and presumably most other skinks and lizards, carry mites routinely in the field. *Neguvon*® dissolves readily in water; we prepare a solution of 1/2 a teaspoon (approximately 1.5 g) of powder per four litres of lukewarm tap water. Higher dose rates of two teaspoons per eight litres (Weigel, 1988), and one teaspoon per six litres (Harlow, 1990) have been recommended in the past, although we believe the reduced dose in conjunction with the second application in our case is the better treatment. Solutions should be made fresh on each occasion. Animals are placed in the solution in a container deep enough for them to be able to swim, and are encouraged to swim for about 30 seconds. We further ensure the head is also treated by pushing the animal briefly under the solution. Animals are then left in a container lined with paper and provided with a heat source for thermoregulation for an hour before being returned to fresh cage. At the same time, all cages are treated as potentially "infected"; all cages are emptied and substrates disposed of. Cages are thoroughly washed and sprayed with a *Neguvon*® solution of the same concentration as used for dipping. Walls and shelving are similarly sprayed. Monitoring the captive population for outbreaks after initial treatment is **vital**; mites are capable of rapid reproduction; for example, the

common snake mite, *Ophionyssus natricis*, lays up to 90 eggs at a time, (Mader, 1989) with a complete life cycle of between 13 – 19 days (Watharow & Reid, 2002).

Restrictions, use and availability

This product should not be used on reptiles that are shedding, or that have recently shed as significant negative effects can result. Extreme care should be taken using this product on, or near, juvenile lizards; dipping is not advised. As an alternative, newspaper or cloth bags can be soaked in a very weak solution of *Neguvon*® and allowed to dry. Dried newspapers can be placed in juvenile cages, or juveniles placed in cloth bags for several hours. If females (and the laboratory) are free of mite problems, offspring will be free also and this treatment becomes unnecessary. While we have had good success with this treatment, we caution each researcher/breeder to develop their own protocols that have been tested on a small group of animals before large-scale treatment.

At present, *Neguvon*® is available "over the counter" in Australia, albeit in relatively large quantities (400 g) because of its typical use in primary production. It should be noted that this product has largely been replaced by other products and may not be commonly found. Care should be taken when using this product: it should only be used when wearing rubber gloves, protective clothing, protective face wear and with care not to swallow or inhale the powder (special restrictions also apply to pregnant women). Upon conclusion of treatment, hands and arms should be washed with warm soapy water, and all equipment used thoroughly washed. Additional safety warnings and first aid advice can be obtained off the packaging and should be read before commencing first treatment.

Summary

This study demonstrates the effective use of Neguvon® in controlling mites in small gravid skinks, but further highlights the importance of monitoring captive populations for mite infestations and the importance of using follow-up treatments several weeks after the initial treatments to ensure complete control. It also highlights the potential for physiological studies to be confounded by the effects of parasite loads. In addition, we believe this to be one of the first reports implicating a native mite species in problems associated with captive reptiles.

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References

- BLOMBERG, S. 1995. Lizards with mites in their pockets. ANH(Autumn): 9-10.
- DOMROW, D. 1985. Species of *Ophionyssus* Megnin from Australian lizards and snakes (Acari: Dermanyssidae). Journal of the Australian Entomological Society. 24: 149-153.

-
- DOMROW, D., HEATH, A. C. G. AND KENNEDY, C. 1980. Two new species of *Ophionyssus* (Acari: Dermanyssidae) from New Zealand lizards. *New Zealand Journal of Zoology* 7: 291-297.
- ESPINOZA, R. E., TRACY, C. R. AND TRACY, C. R. 1998. A safe, single-application procedure for eradicating mites on reptiles. *Herpetological Review* 29(1): 35-36.
- HARLOW, P. (1990). Reptiles. Care and Handling of Australian Native Animals. S. Hands, Surrey Beatty and Sons Pty Ltd, *in association with* Royal Zoological Society of N.S.W.: 41-52.
- MADER, D. R. 1989. Herpetological Medicine: Mites and the Herpetologist. *The Vivarium* 1(4): 27-31.
- MADER, D. R. (1996). Acariasis. Reptile Medicine and Surgery. D. R. Mader, W.B Saunders Company: 341-346.
- MAHANNEY, P. A. 1995. A simple method of mite control. *Herpetological Review* 26(3): 140-141.
- MONTANUCCI, R. R. 1997. A safe procedure for mite eradication. *Herpetological Review* 28(1): 38-39.

OPPLIGER, A., CELERIER, M. L. AND CLOBERT, J. 1996. Physiological and behaviour changes in common lizards parasitized by haemogregarines. *Parasitology* 113: 433-438.

SORCI, G. AND CLOBERT, J. 1995. Effects of maternal parasite load on offspring life-history traits in the common lizard (*Lacerta vivipara*). *Journal of Evolutionary Biology*. 8: 711-723.

SORCI, G., CLOBERT, J. AND MICHALAKIS, Y. 1996. Cost of reproduction and cost of parasitism in the common lizard, *Lacerta vivipara*. *OIKOS* 76: 121-130.

SORCI, G., MASSOT, M. AND CLOBERT, J. 1994. Maternal parasite load increases sprint speed and philopatry in female offspring of the common lizard. *The American Naturalist* 144(1): 153-164.

WAPSTRA, E., SWAIN, R., JONES, S. M. AND O'REILLY, J. M. 1999. Geographic and annual variation in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Australian Journal of Zoology* 47: 539-550.

WATHAROW, S. AND REID, A. 2002. The introduced snake mite *Ophionyssus natricis* on wild populations of eastern blue tongue lizards (*Tiliqua scincoides*). *Herpetofauna* 32(1): 26-29.

WEIGEL, J. (1988). Care of Australian Reptiles in Captivity, Reptile Keepers Association.

Appendix 2

Effect of temperature on induction of parturition by exogenous AVT in *Niveoscincus ocellatus*: a preliminary investigation

Introduction

Temperature is thought to be the most important proximate environmental factor controlling the timing of reproduction in reptilian species (Licht, 1984). In viviparous squamates, parturition may be delayed for weeks by cold experimental temperatures (e.g. *Niveoscincus metallicus*: Swain & Jones, 2000) or naturally for months over winter (e.g. *N. microlepidotus*: Olsson & Shine, 1998; Olsson & Shine, 1999) after the completion of embryonic development. The ability of some viviparous lizards to delay parturition opportunistically for up to four weeks under cold environmental conditions (Chapter 4, Atkins *et al.* in press; Swain & Jones, 2000) may be mediated by an inhibition of the arginine vasotocin (AVT) response at cooler temperatures. In both *N. metallicus* and *N. microlepidotus*, AVT induces parturition more rapidly at warmer temperatures (22 °C and 28 °C), while the response to AVT is delayed, but not prevented at cooler temperatures (Girling, Jones & Swain, 2002).

This preliminary experiment aims to investigate the influence of temperature upon the endocrine mechanisms that control parturition in a viviparous squamate, the spotted snow skink (*N. ocellatus*). *Niveoscincus ocellatus* is a species exhibiting annual reproduction (Jones, Wapstra & Swain, 1997) that occurs over a wide altitudinal range (Rawlinson, 1974). At low altitudes, females give birth in mid-summer (January), while in the high altitude populations, births occur about one

month later (Wapstra *et al.*, 1999). Especially in the highland populations, females may experience poor weather conditions around the expected date of parturition. I hypothesise that decreasing temperature will delay the response to AVT.

Methods

Forty-one pregnant females were collected from a low elevation population at Orford (42°34'S, 147°52'E) on the east coast (50 – 75 m above sea level) of Tasmania, Australia. The females collected were about two weeks from the expected date of parturition (December), and were housed and fed as for Chapter 5 (Experiment 2).

The experiment was started when the first female gave birth to a healthy live litter. The remaining pregnant females were allocated to one of eight groups ($n = 5$) and were moved to the appropriate temperature-controlled room at least 12 hr before injection. The room temperatures were 6 °C, 15 °C, 22 °C or 28°C, as in an earlier experiment investigating the effect of temperature on induction of parturition in *N. microlepidotus* and *N. metallicus* (Girling *et al.*, 2002). Each female was housed individually in a small transparent plastic container with access to water. No cover or heat-lamp was provided so females could not thermoregulate, and room lighting was provided at a 9:15 hr light: dark regime.

Within each temperature treatment, one group received exogenous AVT (0.35 µg/ g nonpregnant body weight = 2 µg/ 50 µl; acetate salt, Sigma Chemical Company) in lizard Ringer's solution (50 µl), and the other group received lizard Ringer's solution only (same volume). The dose of AVT used in this experiment is based on that which successfully induced parturition in *N. microlepidotus* and *N. metallicus* (Girling *et al.*, 2002).

On the morning of the experiment, females received an intraperitoneal injection of AVT or Ringer's solution, and the time of the injection was recorded. All injections were given between 09:00 hr and 10:30 hr. Females were then checked half-hourly for the first day (until lights out), and then several times a day for the next two days to determine whether parturition had occurred. After completion of parturition females and neonates were weighed and measured (SVL), all females were palpated to determine whether they still retained embryos *in utero*.

Only four females gave birth to their entire litter in response to the injection of AVT; therefore a second injection of AVT was administered to the females three days after the initial injection at a higher dose scaled for the pregnant female weight ($4 \mu\text{g}/100 \mu\text{l} = 0.71 \mu\text{g}/\text{g}$ pregnant body weight), and females were checked to determine whether parturition had occurred for a further three days.

Statistics

Data are presented as means \pm one standard error. All analyses were performed in SAS (v6.12). A difference in mean time (min), from the treatment injection until the first birth (log-transformed), between the treatment groups was analysed using ANOVA, with significant differences between treatment groups identified using a Tukey's test. Differences in maternal mass between treatments were also analysed by ANOVA. Homogeneity of group variances were assessed by studying plots of standard deviations against means, while assumptions of normality were checked by examining plots of standardized residuals against estimated values and the normal probability curves of the residuals.

Results

The majority of females (16/19) receiving AVT gave birth to either one or two neonates, whether in response to the first (lower) and/or the second (higher) dose. Only four out of 40 females gave birth to their entire litter in response to the first injection, although three of these were litters of one (Table 1). Seven females gave birth to a partial litter, with all but one of these completing their litter after the second injection of AVT. Of the eight females that did not respond to the first injection of AVT, one delivered her litter of two, and four gave birth to a partial litter after the second injection. Litter size appears to be related to female responsiveness to AVT, with females that gave birth to their entire litter in response to one injection having small litter sizes and the five females that failed to complete their litter after either the first and/or the second injection, having larger litter sizes. There was no significant difference between the temperature treatments in postpartum mass of the experimental females ($F_{(3,12)} = 1.28$, $P = 0.33$, log transformed).

The small number of females responding at the lower temperatures to the first injection dose allowed only differences in mean time until first birth to be tested between females responding at 22 °C and 28 °C: with no significant difference found ($F_{(1,5)} = 2.37$, $P = 0.18$) (Figure 1). The time until first birth was significantly longer at lower temperatures when measured at the more successful second dose ($F_{(2,7)} = 11.52$, $P = 0.006$): with significant differences ($P < 0.05$) in time between females at 6 °C and 22 °C, and females at 6 °C and 28 °C.

Among the control females, one control female held at 28 °C, gave birth within the first injection period (3 days), and two other control females (also held at 28 °C) within the second injection period (a further 3 days).

Table 1 - Response of females to the first injection of 50 µl, and a second injection of 100 µl. Note reduced sample sizes in second injection, females giving birth to a whole litter on first injection not re-injected. (L = litter size)

First injection				Second injection			
Temperature (°C)	No. females giving birth to whole litter	No. females giving birth to partial litter	Females responding / n	No. females giving birth to remainder of litter	No. females giving birth to whole litter	No. females giving birth to partial litter	Females responding / n
6	0/5	2/5	2/5	2/2	0/3	2/3	4/5
15	2/5 (L = 1, 2)	0/5	2/5	n/a	0/3	1/3	1/3
22	1/4 (L = 1)	2/4	3/4	2/2	1/1 (L = 2)	0/1	3/3
28	1/5 (L = 1)	3/5	4/5	2/3	0/1	1/1	3/4

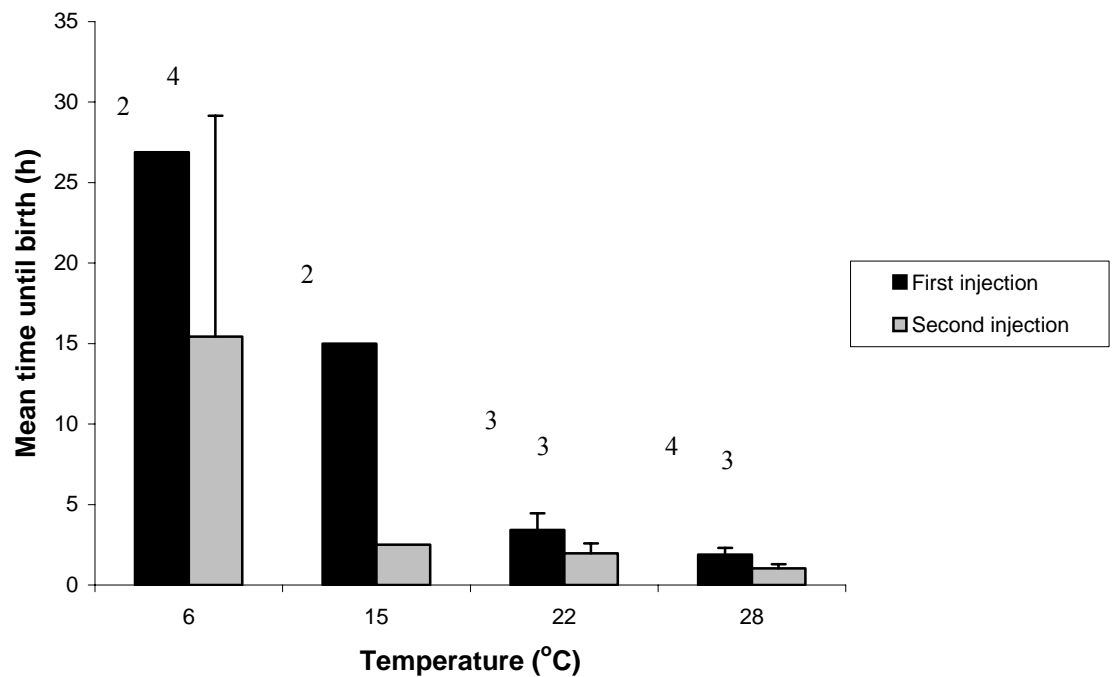


Figure 1 – Mean time until birth of a neonate in a litter after an injection of AVT in *Niveoscincus ocellatus* housed at different temperatures, and includes reactions times to both doses of AVT (reactions to a second dose include females that had already given birth to a partial litter in response to the first dose). Numbers above the columns are sample sizes. Error bars are one standard error of the mean.

For the viable experimental neonates, the mean mass and SVL per litter were 497.3 ± 30.59 mg (4) and 28.3 ± 0.43 mm (4) respectively. For the low viability experimental litters (defined as death on or the day after birth), mean mass and SVL per litter were 481.72 ± 22.40 mg (7) and 27.99 ± 0.24 mm (7) respectively. In addition, three experimental litters were consumed by their mothers, and an additional two litters included dead neonates.

Discussion

In *N. ocellatus*, cooler temperatures significantly delayed, but did not completely inhibit, parturition in response to exogenous AVT, as was the case for *N. metallicus* and *N. microlepidotus* (Girling *et al.*, 2002). While the initial dose of AVT only induced parturition in a minority of females treated, the second dose of AVT, calculated on pregnant female body weight, induced parturition in the majority of *N. ocellatus* females treated.

The short intervals to first birth in response to exogenous AVT in females held at higher temperatures presumably reflect maximal sensitivity of the oviductal tissue to AVT near the species' preferred body temperature (LaPointe, 1977). Females with larger litter sizes were less likely to deliver their whole litter in response to AVT, which may reflect the short half-life of AVT *in vivo* (Harding & Rowe, 2003). However, injection of AVT at 150 or 1500 ng/g did not induce birth of complete litters in any female of *H. maculatus* held at a temperature appropriate to that species, and only 50% of females responded, although the authors suggested that the higher dose is certainly pharmacological (Cree & Guillette, 1991).

The high incidence of stillborn embryos in this experiment presumably reflects the inevitable spread in degree of completion of embryonic development at the time of the experiment (also evidenced by the smaller masses and sizes of the low viability litters): Girling *et al.* (2002) noted similar results in their experiment using *N. metallicus*. Like Bennett (1997) and Girling *et al.* (2002), we noted that many females ate their stillborn offspring, presumably as a mechanism for maternal recycling of nutrients (Blackburn, 1998; Lourdaïs *et al.*, 2005).

These results suggest that temperature does affect the timing of parturition in *N. ocellatus*, and that this may represent a mechanism through which parturition is delayed during adverse environmental conditions. Further experiments (Chapter 5) to test this hypothesis were carried out using the second, higher, dose of AVT.

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References

- Atkins N, Swain R, Wapstra E, Jones SM. in press.** Late stage deferral of parturition in the viviparous lizard *Niveoscincus ocellatus* (Gray 1845): implications for offspring quality and survival. *Biological Journal of the Linnean Society*.
- Bennett EJ. 1997.** A study of gestation in the viviparous skink, *Niveoscincus metallicus* (Honours thesis). University of Tasmania.
- Blackburn D. 1998.** Resorption of oviductal eggs and embryos in squamate reptiles. *Herpetological Journal* **8**: 65-71.
- Cree A, Guillette LJ Jr. 1991.** Effect of β -adrenergic stimulation on uterine contraction in response to arginine vasotocin and prostaglandin $F_{2\alpha}$ in the gecko *Hoplodactylus maculatus*. *Biology of Reproduction* **44**: 499-510.

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- Girling JE, Jones SM, Swain R. 2002.** Induction of parturition in snow skinks: can low temperatures inhibit the actions of AVT? *Journal of Experimental Zoology* **293**: 525-531.
- Harding CF, Rowe SA. 2003.** Vasotocin treatment inhibits courtship in male zebra finches; concomitant androgen treatment inhibits this effect. *Hormones and Behavior* **44**: 413-418.
- Jones SM, Wapstra E, Swain R. 1997.** Asynchronous male and female gonadal cycles and plasma steroid concentrations in a viviparous lizard, *Niveoscincus ocellatus* (Scincidae), from Tasmania. *General and Comparative Endocrinology* **108**: 271-281.
- LaPointe JL. 1977.** Comparative physiology of neurohypophysial hormone action on the vertebrate oviduct-uterus. *American Zoologist* **17**: 763-773.
- Licht P. 1984.** Reptiles. In: Lamming GE, ed. *Marshall's physiology of reproduction*. 4th ed. Edinburgh: Churchill Livingstone. 206-282.
- Lourdais O, Brischoux F, Shine R, Bonnet X. 2005.** Adaptive maternal cannibalism in snakes (*Epicrates cenchria maurus*, Boidae). *Biological Journal of the Linnean Society* **84**: 767-774.
- Olsson M, Shine R. 1998.** Timing of parturition as a maternal care tactic in an alpine lizard species. *Evolution* **52**: 1861-1864.
- Olsson M, Shine R. 1999.** Plasticity of frequency of reproduction in an alpine lizard, *Niveoscincus microlepidotus*. *Copeia* **1999**: 794-796.
- Rawlinson PA. 1974.** Biogeography and ecology of the reptiles of Tasmania and the Bass Strait area. In: Williams WD, ed. *Biogeography and ecology in Tasmania*. The Hague: Junk, W. 291-388.

Swain R, Jones SM. 2000. Facultative placentotrophy: half-way house or strategic solution? *Comparative Biochemistry and Physiology Part A* **127**: 441-451.

Wapstra E, Swain R, Jones SM, O'Reilly J. 1999. Geographic and annual variation in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Australian Journal of Zoology* **47**: 539-550.

