5-2004

Reproductive Biology of Nephrurus and Underwoodisaurus Geckos (Reptilia: Gekkonidae: Carphodactylini)

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Reproductive Biology of *Nephrurus* and *Underwoodisaurus* Geckos (Reptilia: Gekkonidae: Carphodactylini)

By

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Submitted to the Faculty of Science,

The University of Sydney,

In fulfilment of the requirements for the award of the degree of

Doctor of Philosophy in Zoology

May, 2004

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Declaration

I hereby certify that the work described herein has been completed by me and that every effort has been made to acknowledge the assistance of others as specified.

T. Annable

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Dedication

To all those who would love to study a natural history topic in depth but never get the opportunity.

Abstract

This thesis describes the reproductive biology of the endemic Australian gekkonid genera *Nephrurus* and *Underwoodisaurus*. These genera form an important group for investigation because they have both arid and mesic species and occupy both tropical and temperate climates, enabling a comparison of features related to their habitats. Analysis of museum records shows that *Nephrurus* species largely occupy central and northern Australia while *Underwoodisaurus* species largely occupy southern Australia. A morphometric and meristic analysis was carried out on over 1000 alcoholic specimens and over 200 live specimens using up to approximately 50 characters (depending on species, gender and status) of all eleven species (*N. amyae, N. asper, N. deleani, N. laevissimus, N. levis, N. sheai, N. stellatus, N. vertebralis, N. wheeleri, U. milii and U. sphyrurus*). The distinctiveness of the genera *Nephrurus* and *Underwoodisaurus* is confirmed. The distinctiveness of a primitive and a derived group within *Nephrurus* is also confirmed based on radiographic and morphometric data. *Nephrurus deleani* is allocated to the primitive group (includes *N. laevissimus*, *N. levis*, *N. stellatus* and *N. vertebralis*). Also, *N. amyae* and *N. sheai* are allocated to the derived group (includes *N. asper* and *N. wheeleri*) based on phalangeal formula and lack of autotomy plane in the tail. Numerous sexual dimorphisms were demonstrated, including smaller males than females in all species (except *U. milii*), relatively longer limbs in males in most species, relatively longer tails in males and more subdigital lamellae in male *U. milii* and *U. sphyrurus* compared to females. A distinctive ventral escutcheon of enlarged scales occurs in adult *Underwoodisaurus* males. Scattered and distinctive preano-inguinofemoral tubercles are found only in the adult males of *N. deleani, N. laevissimus*, *N. levis* and *N. stellatus*.

Mating (involving a primitive grip by the male) and egg-laying (involving nesting burrow excavation) behaviours are described in detail and are typical of lizards in general. Much of the egg data support the hypothesis that *Nephrurus* eggs are better adapted to aridity than are *Underwoodisaurus* eggs. Egg size relative to maternal size is significantly greater among diplodactyline and eublepharine geckos compared to gekkonine and sphaerodactyline geckos. Relative egg size is also greater among arid *Nephrurus* and *Diplodactylus* species compared to mesic *Underwoodisaurus* and *Diplodactylus* species. Arid *Nephrurus* species have a significantly greater relative

clutch mass compared to mesic diplodactyline species and compared to *Underwoodisaurus* species. Bicone values were determined for *N. deleani. N. levis* and *U. milii* and *U. sphyrurus* egg shape, but values were variable and therefore not as significant in gecko compared to avian eggs. The mean increase in mass during incubation of *Nephrurus* eggs is 24.7 ± 26.4 % of initial mass, which is significantly less than the mean increase for *U. milii* of 42.1 ± 27.1 %. Transmission and scanning electron microscopy and energy dispersive spectrometry of *Nephrurus* and of *Underwoodisaurus* eggshells showed that calcium bicarbonate was distributed largely in the outer region of the shell. Extraembryonic egg contents and egg residues after hatching contain none or insignificant amounts of reducing sugars. Fresh laid *Nephrurus levis* eggs contain approximately 80 % water. After desiccation they contain approximately 53 % protein, 33 % lipid and 7 % ash, with an energy density of approximately 22.2 kJ/g. The eggs of *U. milii* were not significantly different from those of *N. levis* in composition. *Nephrurus levis* and *U. milii* embryos both have an exponential pattern of oxygen consumption during development. The mean energetic cost of embryonic development is 5.82 ± 0.83 kJ/g dry mass for *U. milii* at 25°C and 5.49 ± 0.79 kJ/g at 30°C. This compares with 8.64 ± 2.2 kJ/g dry mass for *N. levis* at 25°C and 8.57 ± 3.4 kJ/g at 30°C. There was no significant difference in the effects of two substrate water potentials (-100 and -450 kPa) on incubation duration at both 25°C and at 30°C. The Q_{10} for duration of incubation of U. milii eggs at 25°C and 30°C was 2.3. Limited egg retention was found in *N. levis*. Measurements of adult testes and ovarian follicles of all *Nephrurus* and *Underwoodisaurus* species indicated large variation in size, even within the breeding season, indicating apparent low synchrony of male and female reproductive systems. Mean testis size increases with snout-vent length throughout life. Histology of testes showed spermatogenesis in all seasons of the year, but probably not mid-winter in southerly distributed species. Sperm storage occurs in *Nephrurus* females, and possibly in *Underwoodisaurus* species. Tails of *Nephrurus* species are multi-functional, being used in alarm responses, feeding, courtship and mating. No territoriality was demonstrated by *Nephrurus* species or by *U. sphyrurus* but adult male *U. milii* (probably the most abundant of all eleven study species) demonstrated strong territorial behaviour particularly in the breeding season.

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Chapter 1

Introduction

1.1 Introduction to Geckos

Geckos are a large and diverse family of lizards with about 1050 species (Uetz, 2003). The geckos (Gekkonidae), together with the Pygopodidae (and possibly the Dibamidae and Xantusiidae, or night lizards) (McDowell and Bogert, 1954; Kluge, 1967a; Moffat, 1973; Wermuth, 1975; Macey, *et al*, 2000; Uetz, 2003), form the Infraorder of gecko-like squamates called the Gekkota (Cuvier, 1817; Camp, 1923; Kluge, 1967a; Macey, *et al*, 1997). Geckos occur on all continents except Antarctica and have a predominantly tropical distribution (Webb, *et al*, 1978; Greer, 1989; Cogger, 2000; Zug, *et al*, 2001).

Because there is still disagreement of what constitutes the Gekkonidae, this thesis adopts a traditional (but still widely accepted) classification, with the geckos (Gekkonidae) consisting of four subfamilies, Sphaerodactylinae, Gekkoninae, Eublepharinae and the Diplodactylinae (Goin, *et al*, 1978; Cogger, 2000). The Gekkoninae (about 732 species) are widespread in the tropics, including Australia, and the Diplodactylinae are centred on Australia and surrounding regions (Bauer, 1990b). Neither the Sphaerodactylinae (about 128 species) nor the Eublepharinae (Eublepharidae according to some authors, *e.g.*, Pough, *et al*, 1998) (about 24 species) are found in Australia.

Geckos range in adult size from 16 mm snout-vent length (SVL) (*Sphaerodactylus ariasiae*), (Hedges and Thomas, 2001) to 370 mm SVL (*Hoplodactylus delcourti*) (Bauer and Russell, 1986; Zug, *et al*, 2001). Most geckos are small (less than 100 mm snout-vent length) and are oviparous (Henkel and Schmidt, 1995; Szczerbak and Golubev, 1996). All geckos have well-developed limbs and are pentadactyl (Goodrich and Winchell, 1889, Pough, *et al*, 1998). Most are also soft-bodied, have large eyes and are nocturnal (Gruber, 1975; Miller, 1984; Cogger, 2000). Unlike lizards in the majority of families of reptiles, most gecko species have an invariant clutch size of one or two eggs (Zug, *et al*, 2001). Geckos are found almost throughout Australia (Storr, 1966; Cogger, 2000). Australian geckos are all nocturnal, oviparous and without movable eyelids (Cogger, 2000). Broadly,

geckos may be divided into climbing species (arboreal or saxicoline) and terrestrial species. The terrestrial species predominate in arid regions and arboreal species in mesic regions. All Australian geckos feed on arthropods, although some species take vegetable matter (Rieppel, 1973; McKeown and Miller, 1985; Gardner, 1988; Cogger, 2000). Tail autotomy occurs in most geckos (Estes, 1983; Cogger, 2000).

The Diplodactylinae consists of two Tribes, the Diplodactylini (five genera and about 55 species) and the Carphodactylini (12 genera and about 68 species, Couper, *et al*, 1993; Kluge, 1993; Bauer and Henle, 1994; Couper and Gregson, 1994; Bauer, 1990b; Bauer, 1994; Hitchmough, 1997; Bauer, *et al*, 1998; Donnellen, et al. 1999; Bauer, *et al*, 2000; Wright, *et al*, 2000; Bauer, Jones, and Sadlier, 2000; Cogger, 2000; Hoskin, Couper, and Schneider, 2003). One species, the gigantic *Hoplodactylus delcourti*, is probably extinct (Bauer and Russell, 1986). There are also several species yet to be described in Australia, New Caledonia and New Zealand (Gill and Whitaker, 1995; Hitchmough, 1997; Jewell, 1997; Couper, *et al*, 2000). The distribution of the Diplodactylini is limited to continental Australia while the Carphodactylini is restricted to the Australasia-New Caledonia-New Zealand region and associated islands (Bauer, 1986; Hitchmough, 1997; Cogger, 2000).

The group of geckos chosen for this study is the carphodactyline genus *Nephrurus* (Figures 1.1 - 1.9) which, at the beginning of this study was considered to consist of seven species (Cogger, 1992). This number later increased to nine species when two new species were described (Couper and Gregson 1994), or eleven species if the genus *Underwoodisaurus* (Figures 1.10, 1.11) is accepted as synonymous with *Nephrurus* (Bauer, 1986; Welch, 1994). There are three generally recognised *Nephrurus* subspecies *N. levis occidentalis* Storr, 1963, *N. l. pilbarensis* Storr, 1963 and *N. wheeleri cinctus* Storr, 1963 (Cogger, *et al*, 1983a; Ehmann, 1992; Cogger, 2000).

In this study, *Nephrurus* and *Underwoodisaurus* are considered a monophyletic group and as separate genera, partly because the synonymy (Bauer, 1986) has not been fully accepted (*e.g.*, Ehmann, 1992; Henkel and Schmidt, 1995; Cogger, 2000; Uetz, 2003), and partly because the morphological and behavioural findings of this study confirm a distinction between the two groups (Chapter 3). It is suggested that the basal trichotomy proposed by

Bauer (1986) will eventually be resolved phylogenetically into two sister groups, the *Nephrurus* and *Underwoodisaurus* genera.

The generic name *Nephrurus* means 'kidney shaped tail' referring to the terminal caudal knob (Russell, and Bauer, 1987a) found on the tails of all *Nephrurus* species (but excluding *Underwoodisaurus* species). The generic name *Underwoodisaurus* is eponymous, referring to G. Underwood, an eminent British zoologist (Ehmann, 1992).

Nephrurus and *Underwoodisaurus* are widespread endemic Australian gekkonid genera (Cogger, 2000; Chapter 2). *Nephrurus* consists of moderate to large geckos (up to 135 mm snout-vent length Cogger, 2000), that are mainly arid or semi-arid species (Figures 1.1 - 1.9). *Underwoodisaurus* is widespread across southern Australia and consists of two moderately large (up to 80 mm snout-vent length), mainly mesic species (Cogger, 2000) (Figures 1.10, 1.11). All diplodactyline geckos lay parchment-shelled eggs that are highly susceptible to desiccation in dry environments (Chapter 5). Hence, the major question addressed in this thesis is, how are *Nephrurus* species capable of surviving in the harsh, arid environment of central Australia when their eggs are so vulnerable?

Following from the above question, I chose to study *Nephrurus* and *Underwoodisaurus* geckos for the following reasons.

1. Most species of *Nephrurus* inhabit arid to semi-arid environments while *Underwoodisaurus* species inhabit more mesic areas, which allows a comparison of the differences in reproductive biology of the two genera.

2. The eggs of all species have soft, flexible shells, which are subject to desiccation. This raises the question of whether the eggs of the arid species differ from those of mesic species.

3. *Nephrurus* and *Underwoodisaurus* species (where known) lay large eggs suitable for energetic studies and chemical analyses.

4. They are all moderate to large species and probably amenable to captive husbandry.

5. Three of the species (*N. laevissimus, N. levis, U. milii*) are well represented in the major Australian museums, allowing adequate morphological study of preserved specimens.

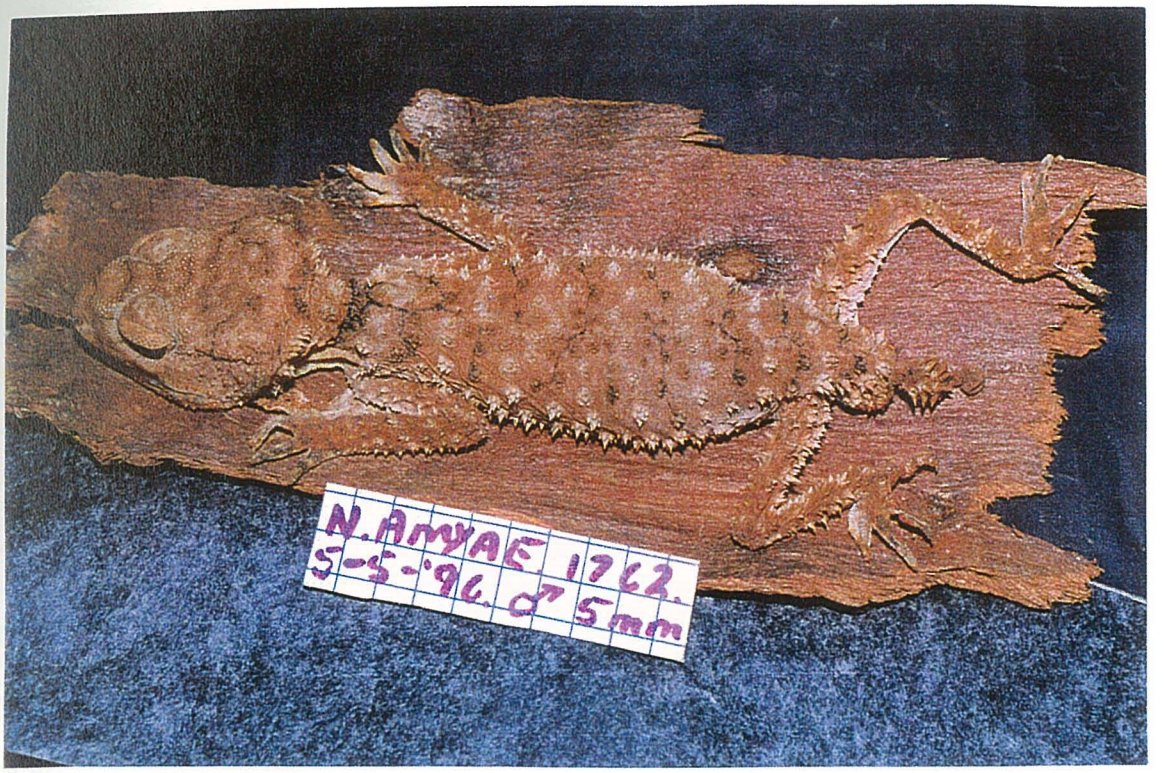

Figure 1.1 Adult male *Nephrurus amyae* Couper and Gregson, 1994, from the Alice Springs area, Northern Territory.

Figure 1.2 Adult female *Nephrurus asper* Günther, 1876, from the Rockhampton area, Queensland.

Figure 1.3 Adult female *Nephrurus deleani* Harvey, 1983, from Pernatty Lagoon, South Australia.

Figure 1.4 Adult female *Nephrurus laevissimus* Mertens, 1958, from the Papunya area, Northern Territory.

Figure 1.5 Adult male *Nephrurus levis* De Vis, 1886, from the Simpson Desert, Northern Territory.

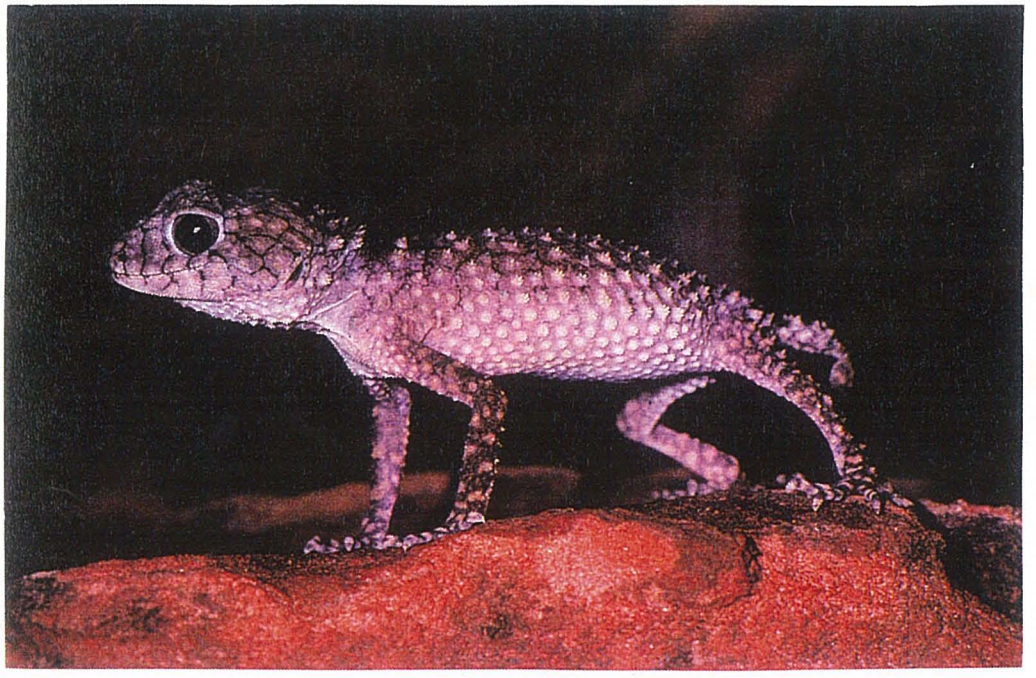

Figure 1.6 Adult female *Nephrurus sheai* Couper and Gregson, 1994, from the Kununurra area Western Australia (photo, D. Bagnall).

Figure 1.7 Young male *Nephrurus stellatus* Storr, 1968, from Middleback Range, South Australia.

Figure 1. 8 Adult female *Nephrurus vertebralis* Storr, 1963 from the Meekatharra area, Western Australia (photo, D. Binning).

Figure 1.9 Adult female *Nephrurus wheeleri* Loveridge, 1932 from the Meekatharra area, Western Australia.

Figure 1.10 Young adult male (above) and female *Underwoodisaurus milii* (Bory de Saint Vincent, 1823) from the Wagga Wagga area, New South Wales.

Figure 1.11 Adult male *Underwoodisaurus sphyrurus* (Ogilby, 1892) from the Tamworth area, New South Wales.

1.2 Introduction to the Phylogeny of Carphodactyline Geckos

In order to make meaningful interpretations of a comparative study of reproduction it is important to consider the phylogenetic relationship of the species involved. The phylogenetic relationships also need to be correlated with the presumed historical processes that led to the present distributions of *Nephrurus* and *Underwoodisaurus*.

The Tribe Carphodactylini is defined by the presence of several rows of preanal pores (a synapomorphy which, however, is not found in *Nephrurus* or *Underwoodisaurus*) and also by the presence of a short wide nasal process of the maxilla (which is plesiomorphic for the Diplodactylinae) (Kluge, 1967b; Bauer, 1986, 1990b). Carphodactylines also have paired or partially divided (in a few species) premaxillae throughout life (Bauer, 1990b). The four carphodactyline genera most closely related to *Nephrurus* and *Underwoodisaurus* are 1. *Phyllurus* Goldfuss, 1820 (leaf-tailed geckos without preanal pores, 5 species), 2. *Saltuarius* Couper, *et al*, 1993 (leaf-tailed geckos usually with adult male preanal pores, 5 species), 3. *Orraya* Couper et al., 2000 (a leaf-tailed gecko with male preanal pores) and 4. *Carphodactylus* Günther, 1897 (monotypic, the most primitive of the Carphodactylini, with long narrow digits, a slightly compressed tail of intermediate length, weakly

developed preanal organs but also some highly derived features, Kluge, 1967b; Bauer, 1990b; Donnellan et al. 1999). Original tails of *Carphodactylus laevis* and *Phyllurus championae* have a minute swelling at the tip (Kluge, 1965a; Hoskin, Couper, and Schneider, 2003), which, however, may not be homologous to the caudal knobs found in *Nephrurus* species.

The currently accepted phylogenetic relationships are shown in consensus cladograms of Australian padless carphodactyline genera (Figure 1.12) and of the *Nephrurus/ Underwoodisaurus* species (Figure 1.13) (Bauer, 1986). Although the Gekkonidae is widespread, its fossil record is poor and currently no useful fossil material of diplodactylines has been described that could help confirm carphodactyline gecko relationships (Bauer and Russell, 1988b; Whitaker, 1992; Hutchinson and Mackness, 2002).

The relevant phylogenetic nodal characters associated with the *Nephrurus/ Underwoodisaurus* group is shown in Figure 1.12. The *N. asper* group comprises the only known geckos without an autotomic fracture plane in the tail (Bustard, 1967b; Greer, 1989) and contains the largest geckos of the genus *Nephrurus*, measuring up to 137 mm SVL (Chapter 3 and Appendix 1).

Nephrurus species have relatively larger heads, wider bodies and reduced presacral vertebral numbers than *Underwoodisaurus* species (Holder, 1960; Greer, 1989; Chapter 3). All *Nephrurus* species have small spinose subdigital scales (Bauer, 1986b) compared to smooth, rounded, monoseriate (non-scansorial) lamellae in *Underwoodisaurus* species (Cogger, 2000; Chapter 3).

There is a close relationship of the genera *Underwoodisaurus* and *Nephrurus*, with *Underwoodisaurus* being the sister taxon of *Nephrurus* (Bauer, 1986b). Geckos of the two genera are superficially similar in size, general morphology and some behaviour patterns. However, there are also numerous divergent characteristics of the genera, including presence of a distinctive and specialised terminal caudal knob, found only in *Nephrurus* species (Figures 1.1 - 1.9), in contrast to *Underwoodisaurus* species, which have a longer, tapering and pointed caudal extremity (Figures 1.10, 1.11).

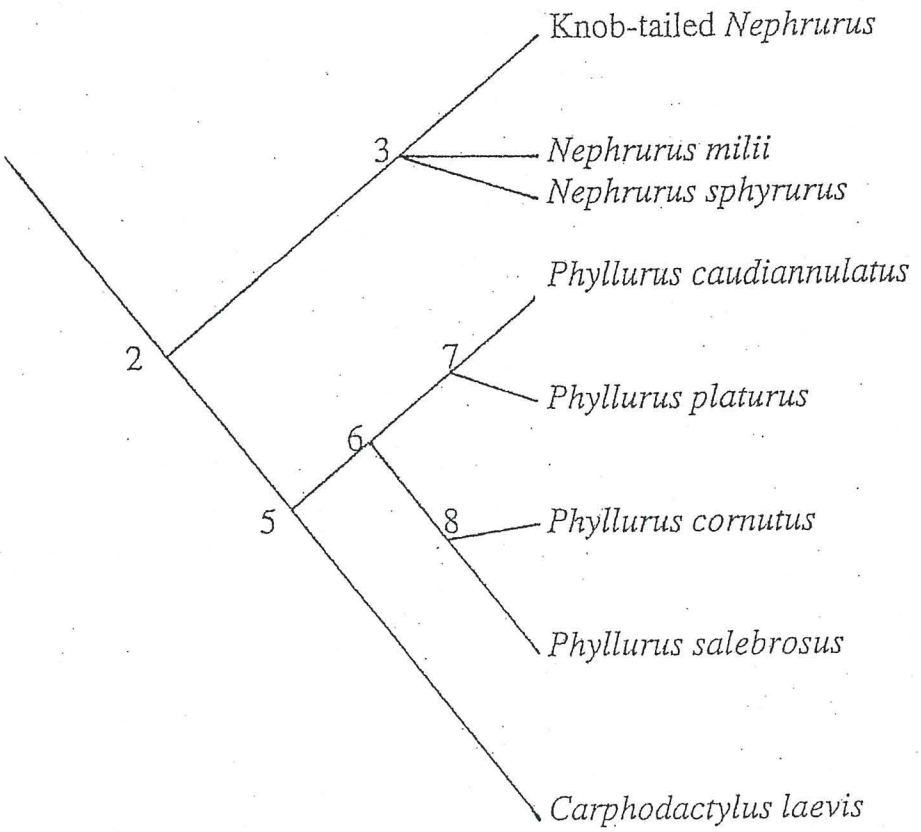

Figure 1.12 Consensus cladogram of Australian padless carphodactyline geckos (Bauer, 1986b).

Node 2 encompasses the genera *Carphodactylus, Nephrurus, Phyllurus, Saltuarius* and *Underwoodisaurus*. The shared derived characters (Bauer, 1986b) are:

1. Premaxillae completely unfused. 2. Parietal short and very broad. 3. Coronoid-dentary suture anterior to dentary surangular suture. 4. Teeth minute and extremely numerous. 5. Hyoid cornu with reduced anteromedial process and enlarged and hooked posterolateral process. 6. Lumbar vertebrae usually 2 (rarely 3). 7. Caudal vertebral centra extremely short. 8. Post-pygal pleurapophyses absent or greatly reduced. 9. Metischial process expanded. 10. Hypoischium slender and elongate. 11. Metatarsal V only slightly hooked. 12. Extrabrillar fringe large, thick, with brown spot on internal face. 13. External ear aperture large and vertical. 14. Dorsal trunk scalation includes tubercles surrounded by rosettes of scales. 15. Preanal organs absent. 16. Cloacal spurs consisting of clusters of conical scales. 17. Tail short and pyramidal. 18. Dorsal tail scalation spinose.

Node 3. (Figures 1.12, 1.13) This node unites *Nephrurus* and *Underwoodisaurus*. The shared derived characters (Bauer, 1986b) are:

1. Zero or one inscriptional ribs. 2. Sternum short and narrow. 3. Clavicular fenestrae very large. 4. Head large. 5. Skin bearing rosettes around tubercles (sic). 6. Digits generally short. 7. Regenerated tail short and bulbous.

(N.B. Node 6. now includes *Saltuarius* and *Orraya* Couper, Schneider, Hoskin and Covacevich, 2000; Hoskin, Couper and Schneider, 2003)

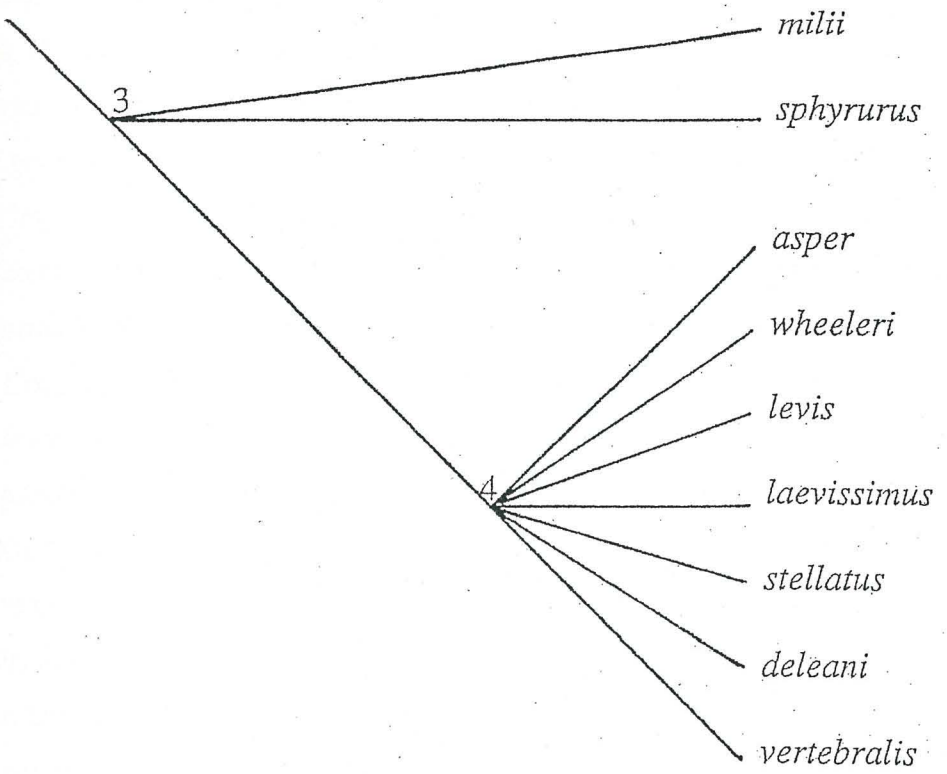

Figure 1.13 Consensus cladogram of *Nephrurus* and *Underwoodisaurus* geckos (Bauer, 1986b).

Node 4. (Figure 1.13) This node includes the knob-tailed geckos of the genus *Nephrurus*. The shared derived characters (Bauer, 1986b) are:

1. Frontal bone approximately as wide as long. 2. Caudal vertebrae fewer than 30. 3. Coracoid process of interclavicle indistinct. 4. Phalangeal formula reduced. 5. Hypoischium extending posteriorly to level of vent. 6. Metatarsals I-IV approximately 2.5 times length of longest respective phalanges. 7. Digit V of pes offset from others. 8. Dorsal colour pattern of three dark bands on head nape and shoulders. 9. Ventral toe scalation spinose. 10. Claws slender at base and slightly decurved. 11. Labial scales only slightly larger than neighbouring scales. 12. Cartilaginous rod of regenerated tail lacking or amorphous. 13. Tail terminating in a small knob.

Underwoodisaurus species also have numerous distinctive osteological, scalation and behavioural differences to *Nephrurus* species (Chapter 3, personal observations).

The *Phyllurus/ Saltuarius* group of leaf-tailed geckos is widely distributed along the mesic eastern coast of Australia. The genus *Phyllurus* currently contains eight species (*P. amnicola, P. caudiannulatus, P. championae, P. gulbaru, P. isis, P. nepthys, P. ossa* and *P. platurus,* Doughty and Shine, 1995; Cogger, 2000; Couper et al., 2000b; Hoskin et al., 2003) of geckos ranging from 75-113 mm maximum SVL, with depressed heads and bodies, long legs, spiny scalation, no preanal pores and similar general biology (what little is known) (Greer, 1989; Ehmann, 1992; Cogger, 2000; Hoskin et al., 2003). The genus *Saltuarius* currently contains four species (*S. cornutus, S. salebrosus, S. swaini,* and *S. wyberba,* Couper, *et al*, 1993; Couper, *et al*, 1997). Geckos of the genus *Saltuarius* range from 110-160 mm maximum SVL, have a crenated marginal flange on original tails and the presence of preanal pores in adult males of two of the four species (Couper, *et al*, 1993; Cogger, 2000). Recently a new monotypic genus *Orraya* Couper et al., 2000 was erected to accommodate the more divergent leaf tailed gecko *O. occultus* (formerly *S. occultus* Couper, Covacevich and Moritz, 1993). *Orraya occultus* reaches a maximum size of 108 mm SVL and has preanal pores in adult males, (Couper et al., 2000b). Because of their close relationship to *Nephrurus* and *Underwoodisaurus*, some data on reproduction and eggs of *Phyllurus, Saltuarius* and *Orraya* species are included in this study (Chapter 5).

The presence of the primitive *Carphodactylus laevis* in northeastern Australia, together with the presence of a larger number of carphodactyline species in eastern and northeastern coastal Australia suggests that the origins of the group may be in north eastern Australia (Kluge, 1965a; Bauer, 1990b).

The close phylogenetic relationship of *Nephrurus* and *Underwoodisaurus* suggests that there would be increased phylogenetic constraint on the results of a comparative study (i.e. fewer phylogenetic differences expected). Also the similarity in adult sizes of a number of the species involved (e.g. *N. levis* and *U. milii*) would minimise any variation due to allometric factors.

1.3 Outline of Aims

Geckos have a more limited range of reproductive modes than other reptiles (Porter, 1972; Bellairs and Cox 1976; Pough, *et al*, 1998; Henkel and Schmidt, 1995; Doughty, 1996; Chapter 4). The majority of geckos, including all Australian species, lay eggs (Pough, *et al*, 1998; Cogger, 2000). Reproductive strategy is correlated with both reproductive and some aspects of somatic morphology (Greer, 1989; Pough, *et al*, 1998; Zug, *et al*, 2001); therefore, I analysed both aspects of morphology to determine the presence of any sexual dimorphisms. I predicted that a variety of reproductive strategies would be accompanied by a variety of morphological (and behavioural) dimorphisms, and that analysis of sexual dimorphisms would provide increased understanding of reproductive strategies (Chapters 3, 4).

The fixed clutch size of two eggs in diplodactyline species (Ehmann, 1992; Zug, *et al*, 2001) means that fecundity is limited. This raises the question of how geckos can maintain high population densities with such a low fecundity? One possibility is that geckos have a greatly reduced predation rate *e.g.* because of their nocturnality. Two other hypotheses advanced here are that:

1. female geckos have within-season iteroparity (Chapter 4).

2. fecundity is increased by reason of increased longevity (Chapter 4).

Another variable reproductive character is eggshell type. Among squamates, only the Dibamidae (Obst, *et al*, 1988), gekkonine and sphaerodactyline geckos lay rigid-shelled eggs (Bustard, 1968b; Dunson, 1982; Deeming, 1988; Greer, 1989). In contrast diplodactyline and eublepharine geckos lay flexible- or parchment-shelled eggs (Werner, 1972; Szczerbak and Golubev, 1996; Pough, *et al*, 1998; Cogger, 2000; Chapter 5). Rigid-shelled gecko eggs are almost impervious to moisture loss (Bustard, 1968b; Schwaner, 1980; Dunson, 1982; Thompson and Russell, 1999b; Chapter 5), suggesting that the Gekkoninae and Sphaerodactylinae are well adapted for reproduction in arid habitats (Chapter 5). Conversely the soft, flexible-shelled eggs (Miller, 1984; Seufer, 1991; Kaverkin and Orlov, 1998) of eublepharine and diplodactyline species desiccate rapidly if exposed to dry air, suggesting that they are less well adapted for xeric conditions (Chapter

5). Analysis of the distributions of the species densities of the Diplodactylinae and Gekkoninae, which are both widely distributed over much of Australia (Chapter 2), provides information on their aridity-adaptation and on their bioclimatic requirements which leads to better understanding of their reproductive strategies.

The majority of Australian geckos lay flexible-shelled eggs, including many species that inhabit arid areas, suggesting that factors such as the choice of egg deposition site are critical to embryonic survival (Muth, 1980; Chapter, 5). This raises the question as to what strategies *Nephrurus* geckos adopt to minimise the risk of egg desiccation. Three hypotheses following on from this question are:

1. that the eggs of diplodactyline geckos from arid environments are more desiccation resistant than the eggs of those from mesic environments (Chapters 5, 6).

2. that because an increased ratio of egg mass to surface area confers protection against aridity, the relative clutch mass will be greater in arid than in mesic species (Chapters 5, 6).

3. that because larger females will be able to lay larger eggs the female: male size ratio will be greater in arid than in mesic species (Chapter 3).

Because arid diplodactyline species will not always be able to find egg deposition sites of adequately high humidity, selection is likely to favour any variation in maternal behaviour or egg morphology (or function) that contributes resistance to desiccation.

The shapes of diplodactyline and gekkonine eggs are different. Most (but not all) gekkonine species' eggs are close to spherical (*e.g.*, Loveridge, 1947; McKeown and Miller, 1985; Seufer, 1991; Douglas, 1992; Szczerbak and Golubev, 1996) and diplodactyline eggs are comparatively more elongate (*e.g.*, Minton, 1966; Bustard, 1967c; Annable, 1992; Seipp and Klemmer, 1994; Sonnemann, 1995; Staniszewski, 1995; Couper, 1996; Rogner, 1997; Tremper, 1999). I compared eggs from the two groups to quantify the extent of this difference to help understand the two reproductive modes. I predicted that the minimum, mean and maximum size of elongate (diplodactyline) eggs would be larger than the minimum, mean and maximum size of the more spherical (gekkonine) eggs, partly because spherical eggs are better able to withstand desiccation-generated forces than elongate eggs,

and do not need to be large to be desiccation resistant and also, because the larger diplodactyline eggs would be more desiccation resistant because of their size (Chapter 5).

The availability of water is critical to the survival of desert animals, including eggs (Muth, 1980; Packard, M. J., *et al*, 1980). Thus, I investigated the effect of different substrate water potentials on embryonic development (Chapter 6). Mean ambient temperature is also of major significance in the development and ecology of poikilothermic organisms (Heatwole, 1987), so I also investigated the effect of different incubation temperatures on embryonic development (Chapter 6).

Embryonic birds and reptiles vary in their patterns of oxygen consumption during incubation, which in some cases can be linked to life history strategies (Thompson, 1989). I investigated the energetics of embryonic development by measuring rates of oxygen consumption and egg carbohydrate, lipid and protein contents, to provide information on gekkonid reproductive strategies (Chapter 6).

The major aim of this research is to determine the characteristics of reproduction in *Nephrurus* geckos that enable them to thrive in arid conditions and make comparisons with reproduction in mesic *Underwoodisaurus* geckos. A holistic approach has been adopted to provide information on both genera because of the paucity of gekkonid physiological and reproductive data available in the literature. This approach gave rise to the following secondary aims:

1. To determine the major life-cycle characteristics of the geckos, particularly as they relate to reproduction, so that comparisons can be made between arid and mesic species (Chapters 2, 4).

2. To describe the major morphometric characteristics of the geckos, particularly in relation to sexual dimorphisms, so that reproductive strategies can be better understood (Chapter 3).

3. To describe the functional morphology of gecko eggs and eggshells, so that the relationship between eggshell and environment can be better described (Chapter 5).

4. To characterise the major physiological characteristics of the eggs that may enable them to develop in arid conditions (Chapters 5, 6).

5. To relate the morphological, biochemical and physiological characteristics thus determined, to the behavioural and ecological characteristics of the geckos (Chapters 3, 4, 5, 6).

Chapter 2

Distributions

2.1 Introduction

The pan tropical distribution of the highly heterogeneous and speciose gekkonine geckos and their limited radiation in Australia suggests that they did not originate in Australia (Kluge, 1967a). In contrast, the restriction of diplodactyline geckos to the Australia, New Zealand, New Caledonia, Loyalty Islands region suggests that they originated in this region (Kluge, 1965a; Cogger and Heatwole, 1981; Bauer, 1990b). Knowledge of the present distributions and habitats (Appendix 5) of *Nephrurus* and *Underwoodisaurus* will help in the understanding of their reproductive strategies. At least one species of the genus *Nephrurus* is found throughout most of mainland Australia, with the exception of Victoria, most of NSW, coastal eastern Australia and the SW of Western Australia (Wilson and Knowles, 1988; Ehmann, 1992; Cogger, 2000). Most parts of Australia support only one or occasionally two (rarely three) species of *Nephrurus* (Cogger, 2000). Arid terrestrial geckos tend to have low population densities and in central Australia have biomass densities of about 100 to 330 gHa^{-1} (Henle, 1988). The low species densities and low biomass densities suggest that *Nephrurus* forms a minor component of the arid herpetofauna. The genus *Underwoodisaurus* is also found in all mainland states, but has a predominantly southern distribution (Ehmann, 1992; Cogger, 2000). The broad-based distribution maps (Wilson and Knowles, 1988; Ehmann, 1992, Cogger, 2000) show that *Nephrurus* and *Underwoodisaurus* may be found over about 90% of the Australian mainland area, with only the extreme south-western, extreme south-eastern and coastal eastern Australia excluded.

Because of the small numbers of species involved, an analysis of distributions of *Nephrurus* and *Underwoodisaurus* species alone is not adequate for determination of any fundamental differences in their habitat requirements. Consequently, a comparative study of distributions of the two gekkonid subfamilies represented on the Australian mainland

(Gekkoninae, with about 25 species and Diplodactylinae, with about 80 species) was made. Thus a stronger statistical argument could be made regarding habitat requirements.

Species density is defined here as the number of gecko species that occur in a given area. I used published distribution maps to determine gecko species densities (Bauer, 1986; Wilson and Knowles, 1988; Ehmann, 1992; Bauer and Henle, 1994; Couper and Gregson 1994; Cogger, 1996; Cogger, 2000). Knowledge of species density over a given area (when correlated with phylogenetic and other regional data) provides extensive information on zoogeography, speciation, suitability of habitat and characteristics of their adaptations to aridity. For example a comparison of gekkonine and diplodactyline species densities can be used to infer preferred habitat in terms of aridity.

The biology and distributions of reptiles are significantly affected by temperature and rainfall (Cloudsley-Thompson, 1971; Heatwole and Taylor, 1987; Autumn, 1993). Therefore, a comparison of distributions with isotherm and isohyet maps will provide information on life history requirements. Australian deserts are not hyperarid as are parts of the Sahara, Saudi and Gobi deserts (Bradshaw, 1986); nevertheless, the arid zones of central Australia present a considerable stress for the organisms inhabiting the region, with extremes of humidity and temperature (Cogger, 1984). The rainfall averages over the *Nephrurus* - *Underwoodisaurus* species distributions vary from about 100 mm per year in parts of central Australia to greater than 1,600 mm in north Queensland (Castles, 1992). In addition, the weather patterns vary dramatically from warm monsoonal summer rains in the north to more aseasonal rains or cold winter rains further south. Also, there are moderate to severe frosts in the south of the continent every winter (Foley, 1945; Bureau of Meteorology, 2002), but southern geckos probably avoid the frosts by taking refuge in deep burrows for the dormant stage. Furthermore, given that *Nephrurus* - *Underwoodisaurus* geckos are nocturnal, foraging may have to occur at even cooler temperatures than for sympatric diurnal species (Autumn, 1993). A distinctive characteristic of *N. sheai* is that it is found exclusively in a tropical monsoonal zone and might be expected to possess distinctive reproductive habits. Similarly, the distribution of *N. asper*, extending from tropical monsoonal northern Queensland to the arid interior of southern Queensland, suggests a varied life history in the differing localities.

2.2 Methods

A locality is defined as a distribution point with a distinct latitude and longitude. Many of the localities overlap at the mapping scale used in this study, but are adequate for demonstrating species distributions on a broad scale. *Nephrurus* and *Underwoodisaurus* distributions were also generated from locality data for all the *Nephrurus* and *Underwoodisaurus* geckos held in all the major Australian museums, as well as additional locality data accumulated during this study (Appendix 1). Latitudes and longitudes were decimalised so they could be plotted directly on a Mercator projection map of Australia. Doubtful records (except in the case of obvious errors such as marine environments) were not deleted without consultation with the responsible curators.

For species distributions analysis the generally more specific and comprehensive published maps were chosen (Ehmann, 1992) over other less specific, less detailed or less comprehensive maps (*e.g.* Storr, Smith and Johnstone, 1990; Swan, 1990, 1995; Ingram and Raven, 1991; Cogger, 1992, 1996, 2000). These other references were not used unless the distribution of a given species was unavailable in the primary source (Ehmann, 1992). Thus reducing discrepancies, such as would occur at State boundaries.

Species density maps were generated by scaling up the published area distribution map for each species of gecko and superimposing them, one at a time, onto an A3 sized map of Australia. The numbers were then summed, ignoring very small areas of overlap or non-conjunction of areas. The resulting isoclines (or isotaxa lines) were then slightly rounded to produce more readable species density maps. This process was repeated for each of the two subfamilies Gekkoninae (25 species) and Diplodactylinae (80 species). The resulting isoclines were analysed by calculating the average species density at each latitudinal degree across Australia, and the species densities were then plotted against latitude for each subfamily. A second method was adopted in which a transparency was made of a map of Australia to which a 200 x 200 km rectangular grid was added. The transparency was then superimposed over a scaled photocopy of a point-distribution map derived from museum collection data for each species in turn and the numbers of each species occurring in a given square recorded on the grid.

The level of aridity for the distribution of each *Nephrurus*, *Underwoodisaurus* or other gecko species was found by superimposing the area distribution map of each species over an annual precipitation map of Australia showing arid, semi-arid and mesic regions, and determining the location of the centre of (or major portion of) each distribution by eye. An arid region is defined as one receiving less than 300 mm mean annual precipitation and a semi-arid region as one receiving between 300 and 600 mm mean annual precipitation (Gentilli, 1978).

2.3 Results

The point distribution maps for individual species generated mainly from museum records (Figures 2.1 - 2.11) largely confirm the published distributions. The combined distributions of the genera *Nephrurus* and *Underwoodisaurus* also show coverage of about 90 % of the Australian mainland (Figures 2.12 and 2.13). There are however, large gaps in remote northern regions for *Nephrurus* species (Figure 2.12). *Underwoodisaurus milii* has an Australia-wide southerly distribution, with small, probably relictual populations in the Northern Territory and western Queensland. The latitudinal range of individual *Nephrurus* and *Underwoodisaurus* distributions is smaller than the longitudinal range for all but three species (*Nephrurus deleani, N. wheeleri and U. sphyrurus*) (Ehmann and Tyler, 1995; Cogger, 2000).

Of the 80 species of diplodactyline geckos, no *Oedura, Orraya, Phyllurus, Pseudothecadactylus* or *Saltuarius* (26 species) is predominantly arid. However, 25 (31.3%) of diplodactyline geckos are restricted or largely restricted to the arid and semi-arid regions (*Diplodactylus assimilis, D. byrnei, D. elderi, D. fulleri, D. galeatus, D. immaculatus, D. jeanae, D. kenneallyi, D. klugei, D. mitchelli, D. pulcher, D. rankini, D. savagei, D. squarrosus, D. strophurus, D. tessellatus, D. wellingtonae, Nephrurus amyae, N. deleani, N. laevissimus, N. levis, N. stellatus, N. vertebralis, N. wheeleri, Rhynchoedura ornata*). In comparison only six (22.2%) of 27 gekkonine species are restricted or largely restricted to arid regions (*Gehyra minuta, G. montium, G. pilbara, G. punctata, G. purpurascens* and *G. robusta*). The proportions of arid species in each subfamily, by χ^2 contingency test, are not significantly different at P = 0.05. *Nephrurus* species densities are highest in areas of low to very low precipitation, but they also occur in

areas of moderately high or seasonal monsoonal precipitation in the north. Unlike all *Nephrurus* species, *U. milii* is found in a large range of habitats from arid to mesic, although the distributions of *N. deleani* and *N. stellatus* lie wholly within the distribution range of *Underwoodisaurus* (Figures 2.3, 2.7, 2.13).

Nephrurus species distributions range in latitude from about 11°S (*N. asper* near Cape York, Queensland) to about 34 °S (*N. stellatus* in the Eyre Peninsula region, South Australia) (Figure 2.12). *Underwoodisaurus* species range from about 21°S (*U. milii* near Alice Springs, Northern Territory) to about 37 °S (*U. milii* near Bendigo Victoria) (Figure 2.13), as derived from museum distribution data and largely confirmed by published distributions (Wilson and Knowles, 1988; Ehmann, 1992; Cogger, 2000; personal observations). One museum record for *U. milii* at 38 °S was found to be in error, and scattered records for northern Western Australia and northern Queensland (Bauer, 1986) could not be confirmed from museum records.

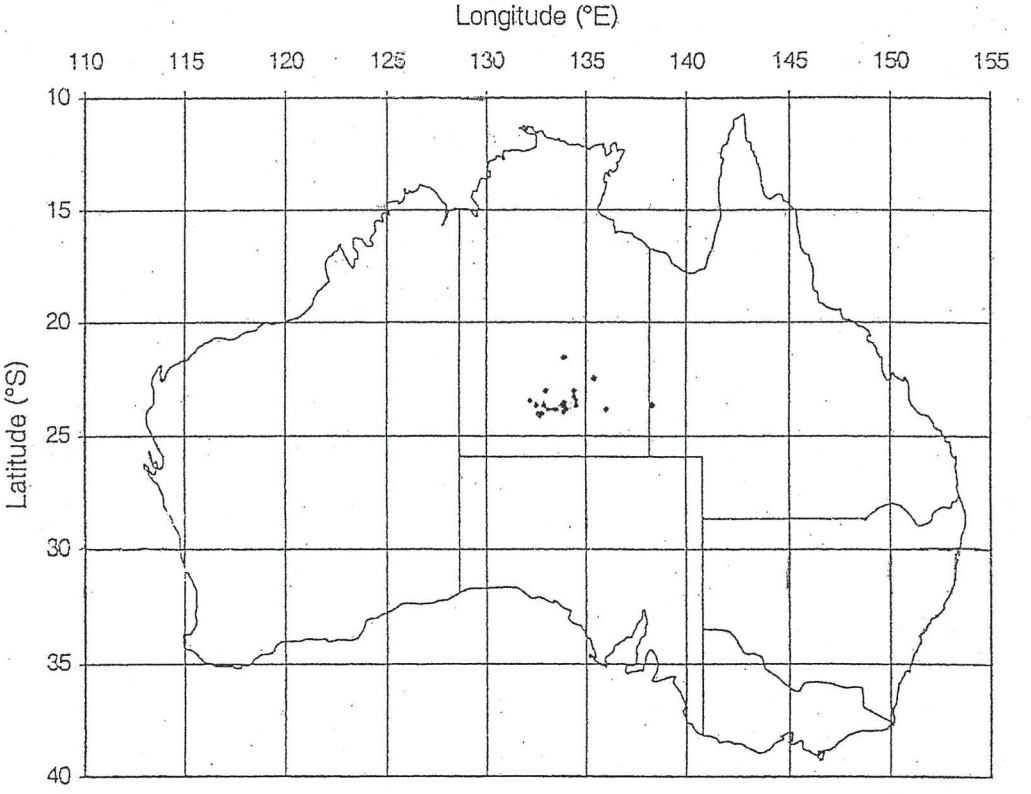

Figure 2.1 Distribution of *N. amyae* (N = 25 localities). This is probably the most arid adapted of all *Nephrurus* species.

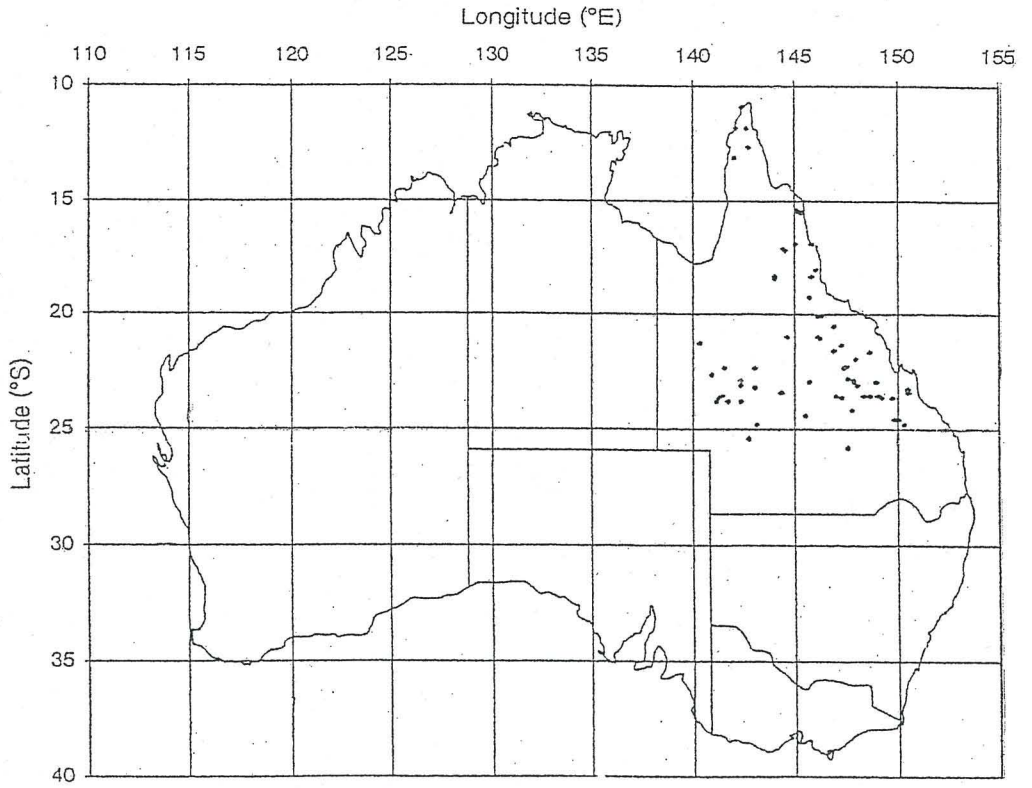

Figure 2.2 Distribution of *N. asper* (N = 66 localities).

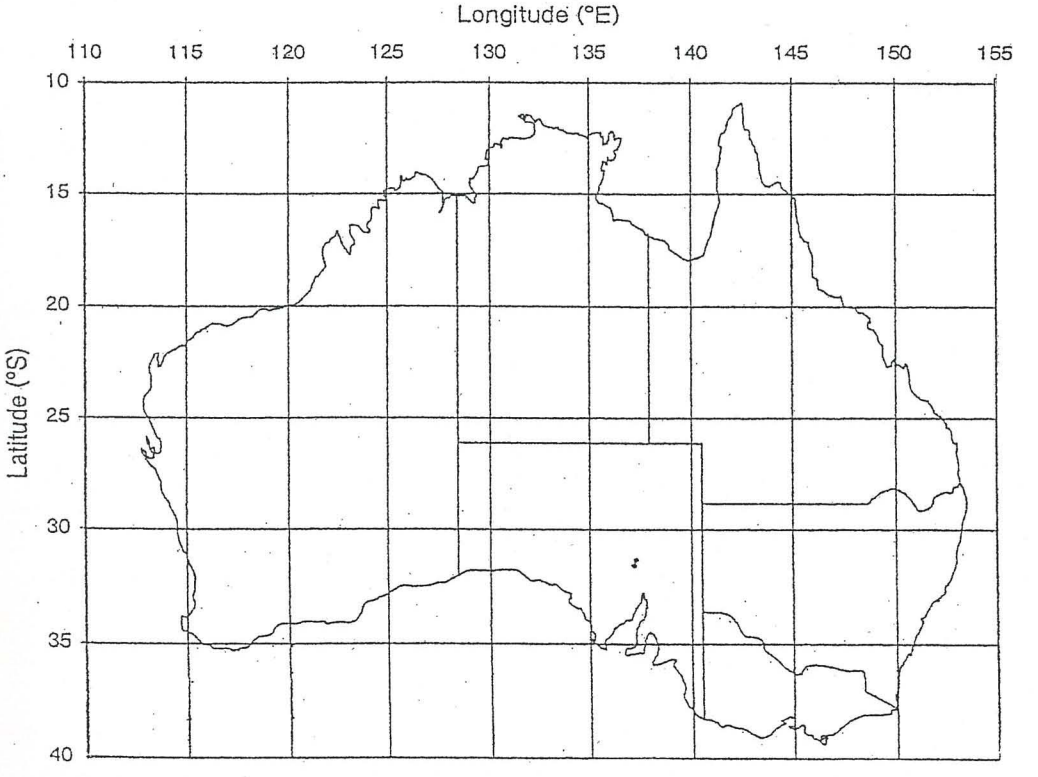

Figure 2.3 Distribution of *N. deleani* (N = 9 localities), the most restricted of the *Nephrurus* species.

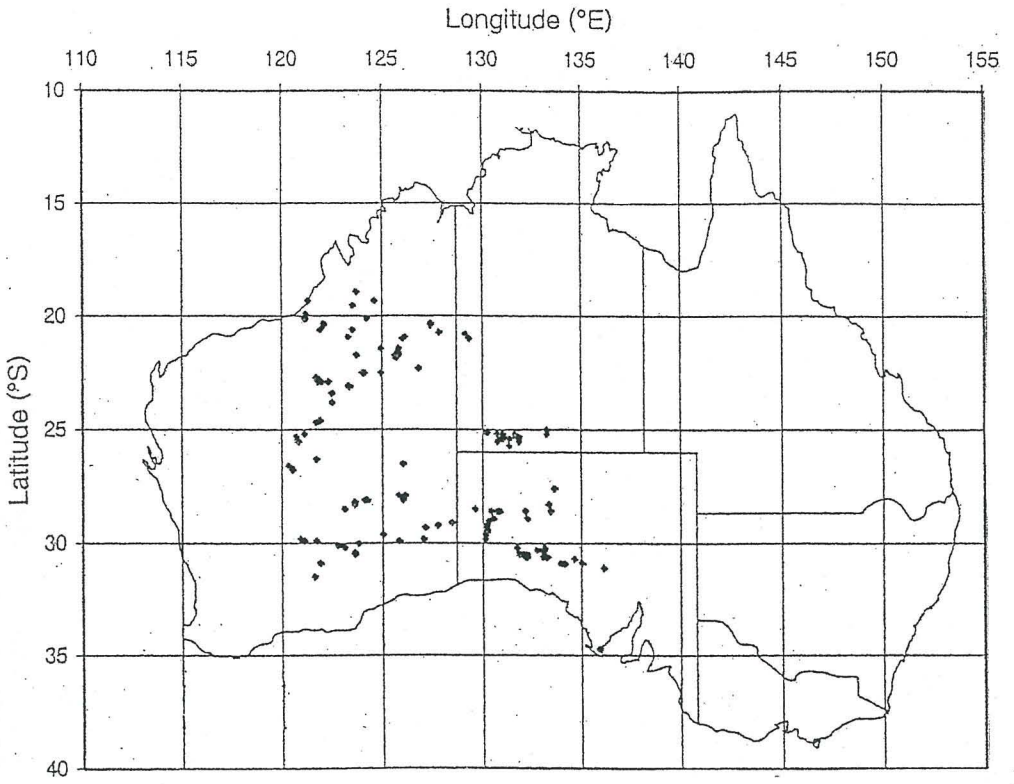

Figure 2.4 Distribution of *N. laevissimus* (N = 130 localities).

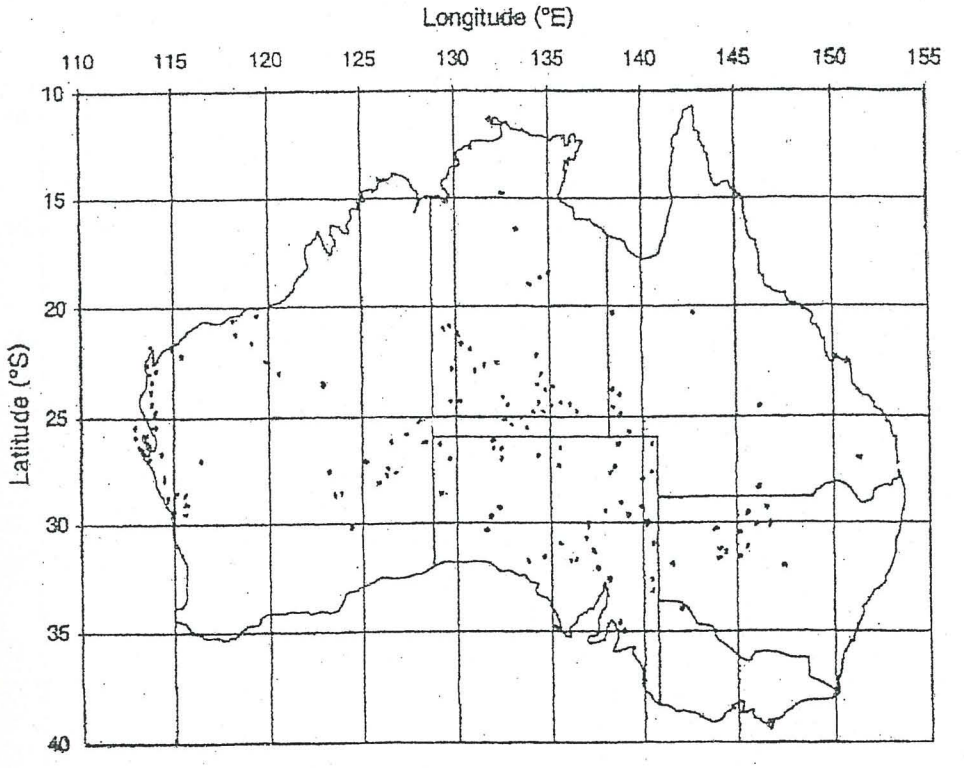

Figure 2.5 Distribution of *N. levis* (N = 488 localities), the most widespread *Nephrurus* species.

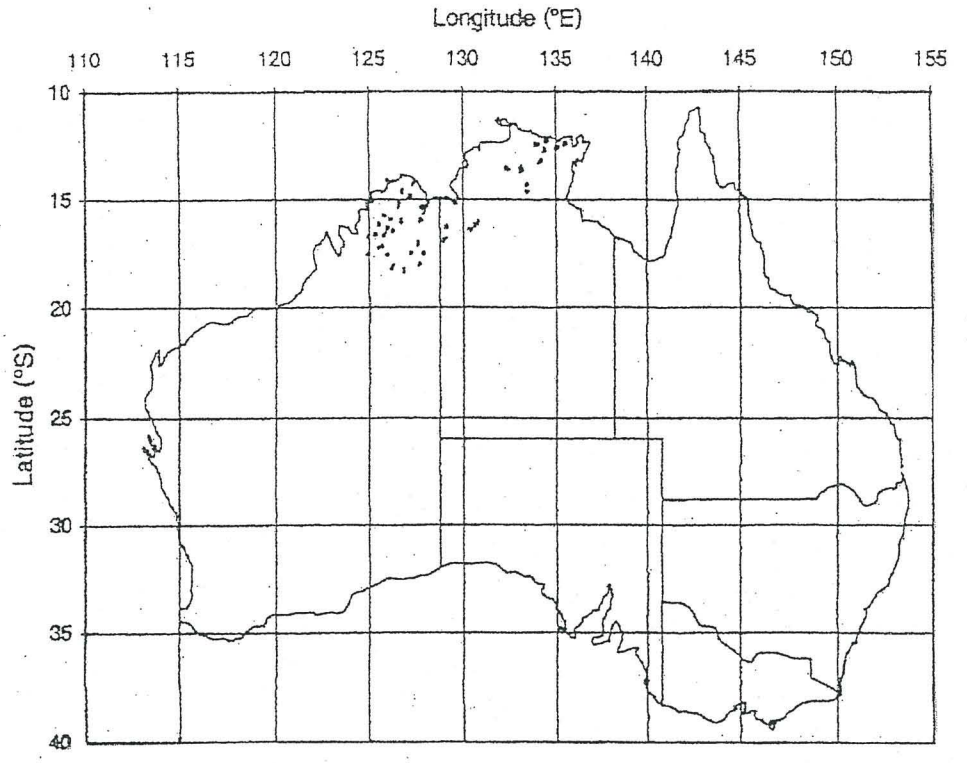

Figure 2.6 Distribution of *N. sheai* (N = 66 localities).

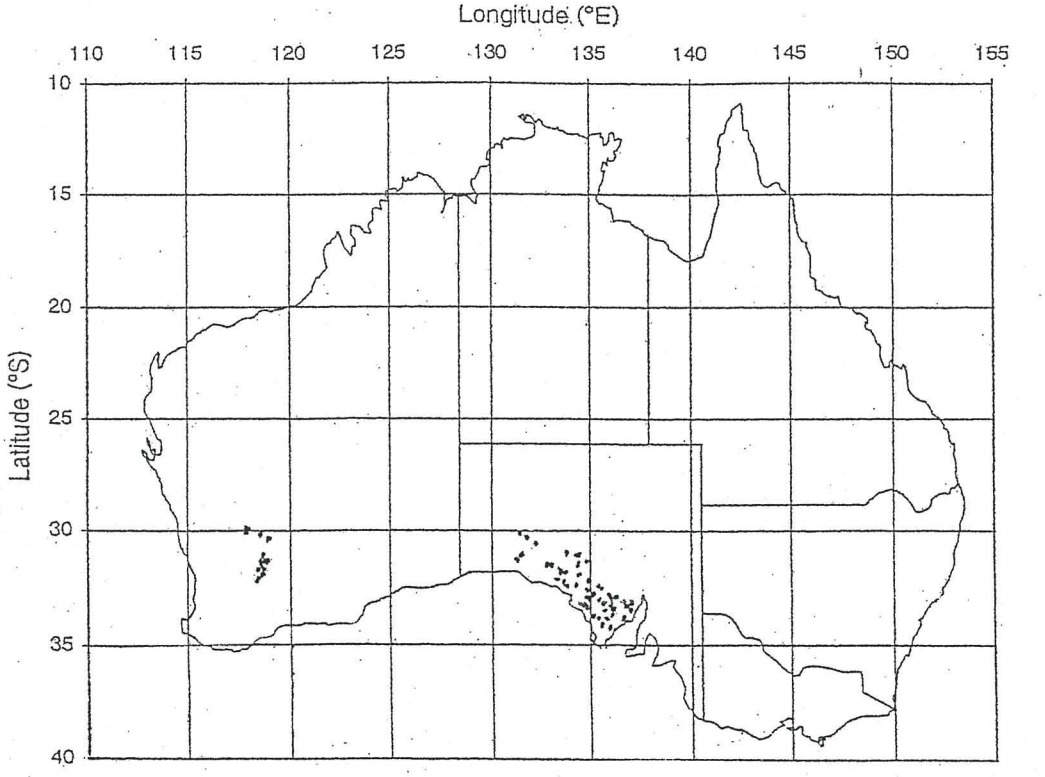

Figure 2.7 Distribution of *N. stellatus* (N = 58 localities).

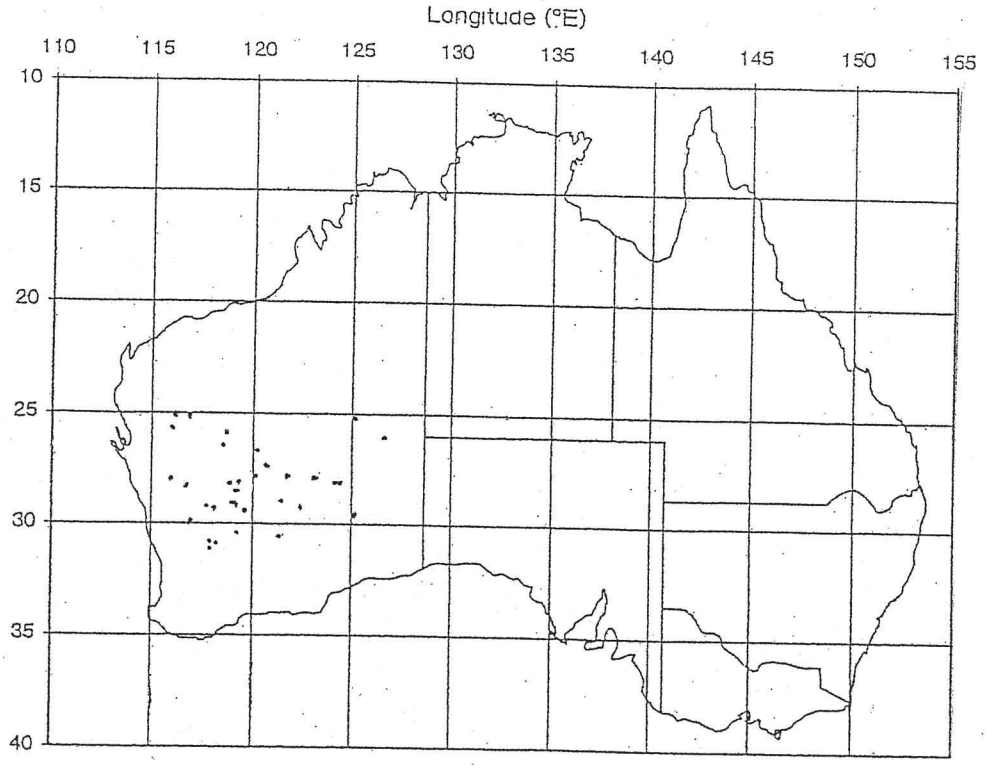

Figure 2.8 Distribution of *N. vertebralis* (N = 39 localities).

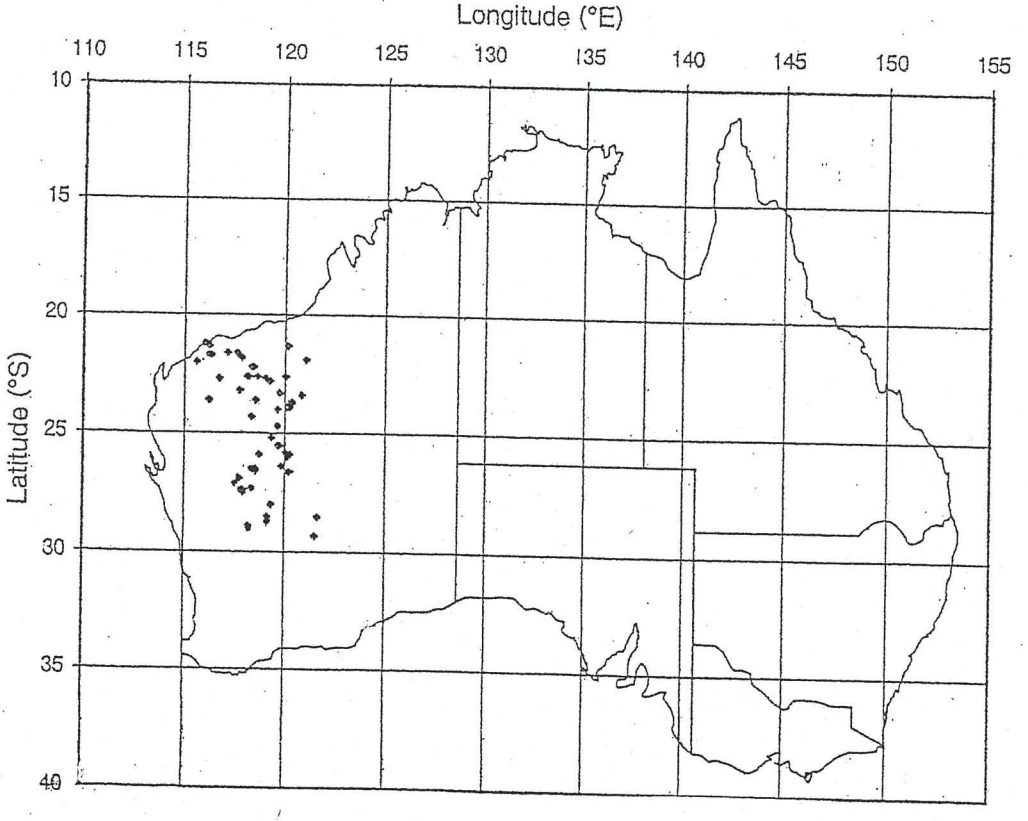

Figure 2.9 Distribution of *N. wheeleri* (N = 56 localities).

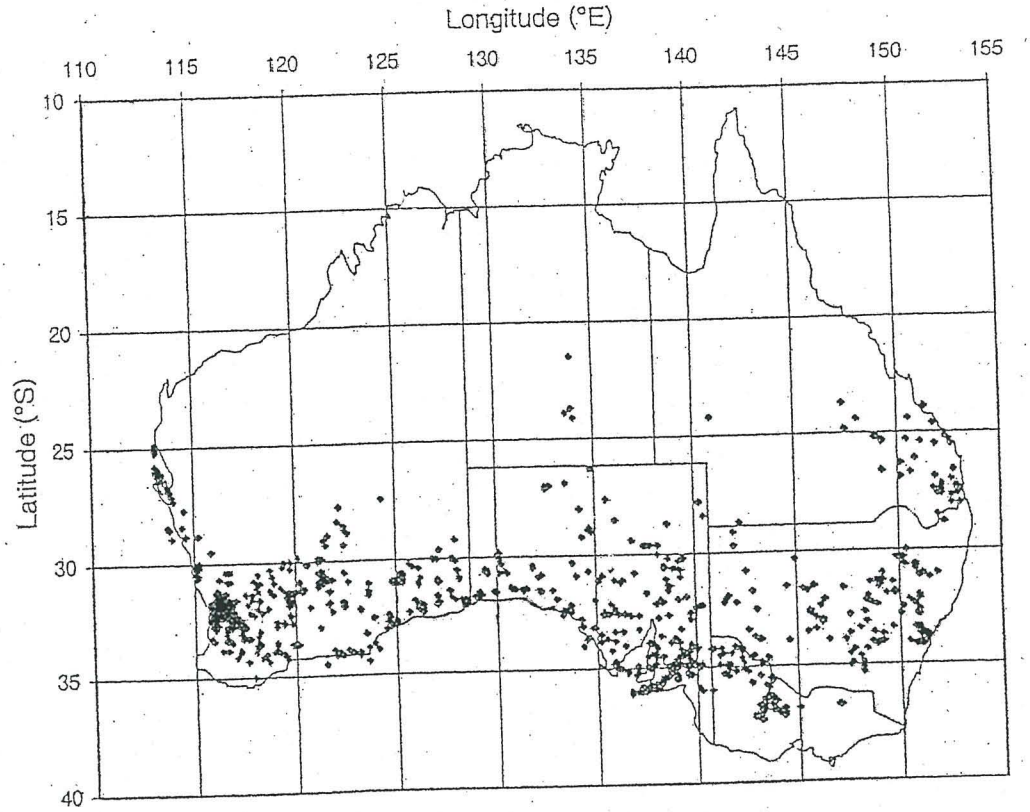

Figure 2.10 Distribution of *U. milii* (N = 791 localities).

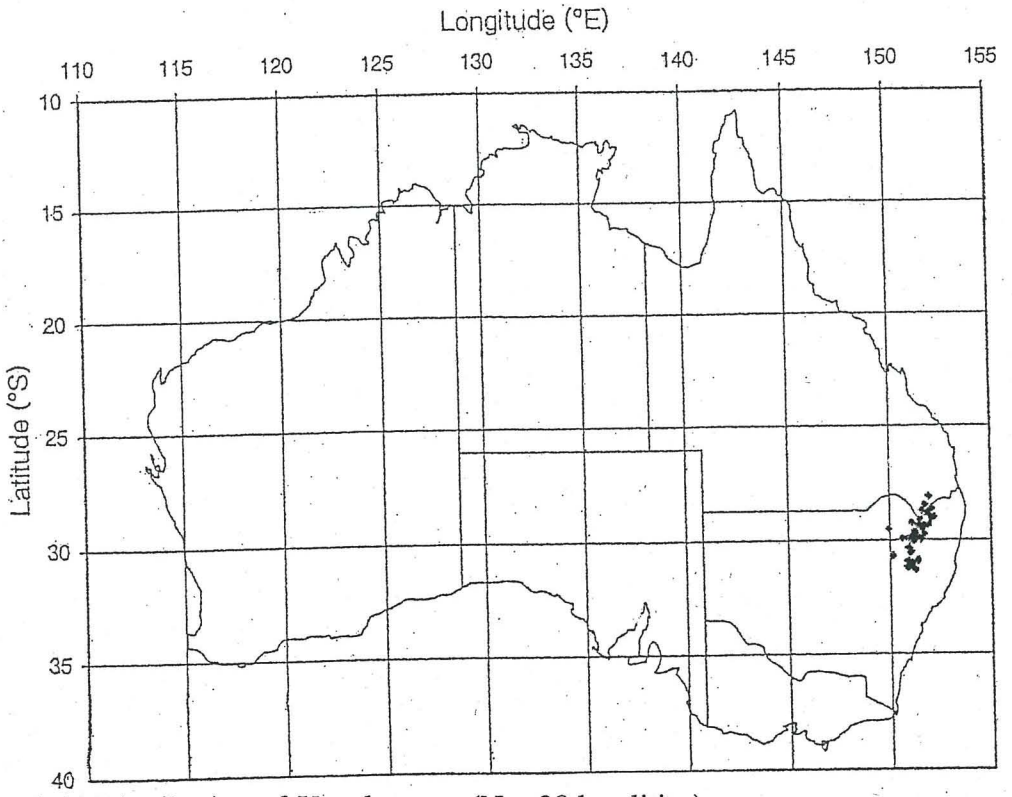

Figure 2.11 Distribution of *U. sphyrurus* (N = 38 localities).

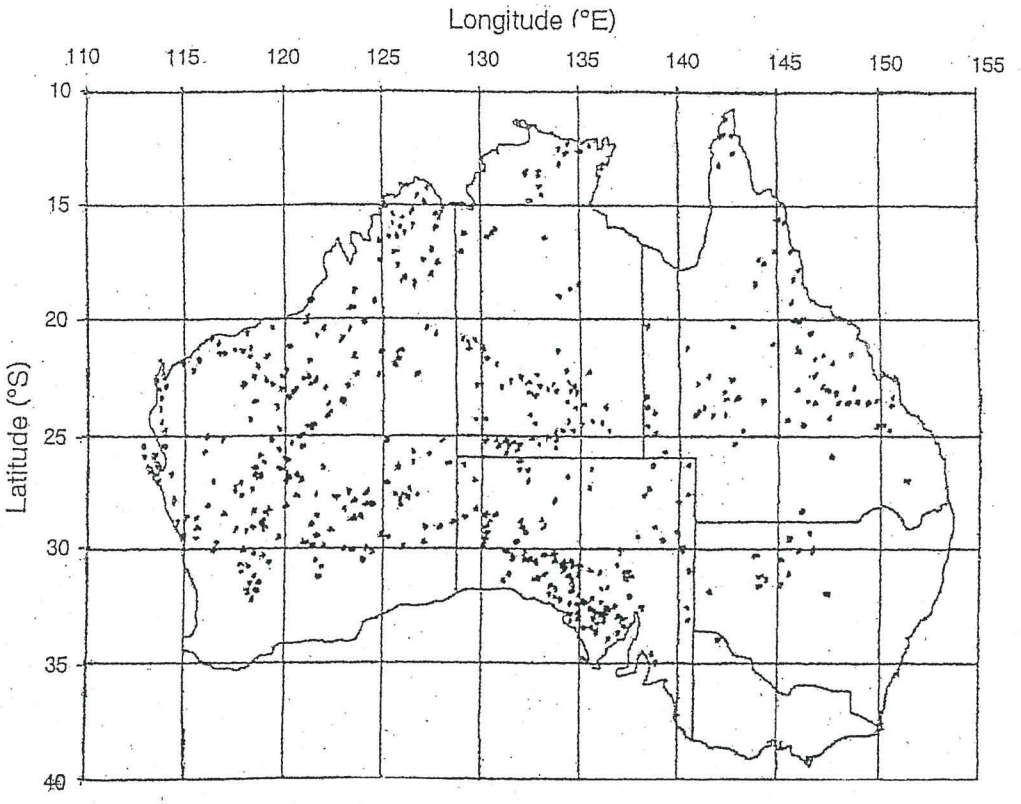

Figure 2.12 Combined *Nephrurus* species distribution. The individual species distributions are largely allopatric, with small areas of overlap (Wayne, 1996).

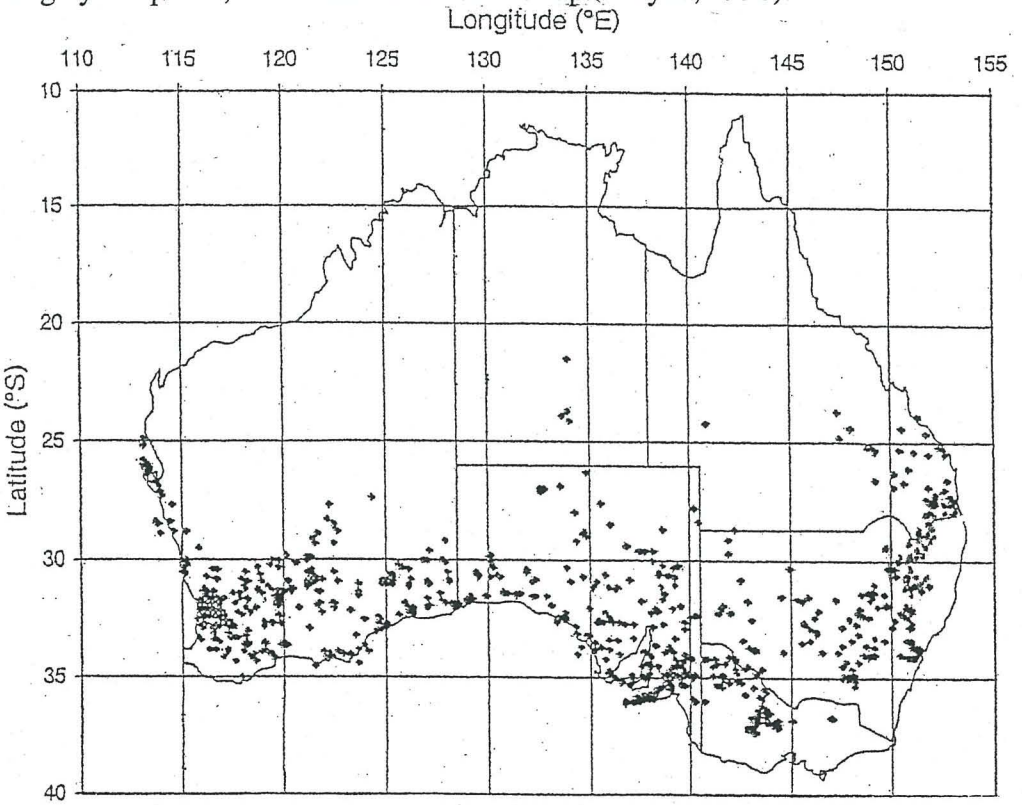

Figure 2.13 Combined *Underwoodisaurus* species distribution. The *Underwoodisaurus* species distributions tend to be more southerly and mesic than *Nephrurus* species (Cogger, 2000).

The Gekkoninae (26 species) is spread over much of the Australian mainland, with zero representatives only in parts of the coastal east and southeast (Figure 2.15). The highest species density occurs in extreme northern Cape York Peninsula (six species). Other areas of moderate species density are found on east-coastal Cape York Peninsula, where there are five to six species. There are also areas of five species in the Pilbara region, Kimberley area and just south of Darwin. Much of central Australia is occupied by three or four gekkonine species.

The Diplodactylinae (80 species) shows a more complex species density distribution (Figure 2.16), with the highest species densities in Central Australia (up to 14 species) and in the Pilbara and central Western Australia regions (12 species). There are also several areas scattered across Australia in all mainland states, except Victoria, with 10 or 11 species. The Diplodactylinae do not extend as far south as the Gekkoninae in both Victoria and Western Australia. Species densities of the Diplodactylinae are greater than for the Gekkoninae in all areas except in northern Cape York Peninsula and the extreme south of Victoria and Western Australia. Nine out of ten areas where the species density exceeds nine are in arid and semi-arid regions (Figures 2.15, 2.16, 2.17).

Comparison of mean species density with latitude shows a large difference between the two subfamilies, even allowing for the supposed recent arrival of the gekkonines in Australia (Kluge, 1967a; Figure 2.17). The variation of the average species density for gekkonine geckos at each degree of latitude across Australia shows a strong inverse correlation (R^2 = -0.899); this compares with a much lower correlation (R^2 = -0.492) for the diplodactylines. The diplodactyline species density reaches zero at 37°S and the gekkonines at 39°S. The gekkonines have their highest species density at 11-12°S whereas the diplodactylines reach their highest species density at 23-25°S. Comparison of the two methods (isocline mapping and 200 km grid) used to determine diplodactyline species density shows similar results, with a maximum density of 14 in each case.

Two species of geckos (*Diplodactylus vittatus* (Diplodactylinae) and *Christinus marmoratus* (Gekkoninae)) have reached the Australian High Country in south-east Australia (Jenkins and Bartell, 1980). Both species range from sea level to elevations up to 1000 m at several localities around Canberra and Cooma.

2.4 Discussion

Recent evidence from chromosomal, immunogenetic and biochemical studies complement the morphological and distribution studies of the past to provide new data on the possible origins of the Gekkonidae in Australia. The Gekkonidae has variously been described as being Gondwanan (e.g. Tyler, 1979) or Laurasian (Cogger and Heatwole, 1981) in origin. The newer evidence suggests that the Gekkonidae is a Gondwana relic but that there have been repeated invasions of mesic geckos from the north (King, 1990). The indigenous origins of the Diplodactylinae and the recent extraneous origins of the Gekkoninae are generally agreed (Kluge, 1967a; Cogger and Heatwole, 1981; Bauer, 1990b). The differences in gekkonine and diplodactyline species density with latitude and also proportion of each group inhabiting arid regions suggests that there are factors involved in their distributions other than time of arrival. There are no deserts in the countries surrounding Australia from where immigrant geckos probably originated. Therefore it is expected that all geckos arriving in northern Australia would be pre-adapted to a tropical mesic climate (Cogger, 1984). However, the Gekkoninae geckos are not restricted to the more mesic regions (as might be expected from their origins) nor are they found mainly in arid regions (as might be expected from a consideration of their egg-shell properties) (compare Figures 2.14, 2.15, 2.16). The concentration of diplodactyline (including *Nephrurus*) species in the arid zones suggests that they are well adapted to aridity and it would be expected that their reproductive and life history patterns would reflect these adaptations (Chapters 3, 4).

The species distribution maps show several factors suggesting that distributions have changed over time. The fact that the most northerly confirmed locality for *Underwoodisaurus milii* in Western Australia is Dorre Island (25°S) suggests that the mainland distribution of this species may have retreated southwards, probably as a result of palaeoclimatological changes. The relictual populations of *U. milii* in the Northern Territory and western Queensland also suggest that the distribution was more extensive in the past. Another putative distribution change is indicated by a gap of approximately 900 km between the Western Australian and the South Australian populations of *N. stellatus*,

suggesting that the distribution of this species has also contracted significantly over time. The fact that there has been very little

morphological divergence between the eastern and western populations suggests a geologically recent separation. The distributional changes in *U. milii* and *N. stellatus* could be explained on the basis of reducing precipitation and increasing aridity in central Australia (Cogger and Heatwole, 1981). The relictual populations of *U. milii* in Central Australia imply that they are surviving close to their environmental limits and also that significant plasticity exists in their reproductive mode.

Gaps in the distribution maps are expected because of inadequate survey work, particularly in remote areas (Figures 2.12, 2.13) and also because of unsuitable habitat. *Nephrurus* and *Underwoodisaurus* would not be expected in dense rainforests, wetlands, cold highlands, gibber deserts, closed sclerophyll forests, intensive farmlands and flood prone areas. In spite of these limitations, the major aspects of the analyses are still valid. The present distribution of species supports speculation that the *Underwoodisaurus/ Nephrurus* dichotomy and subsequent speciation was driven by the Eocene desertification of central Australia, and continued throughout the Miocene period (Bauer, 1986).

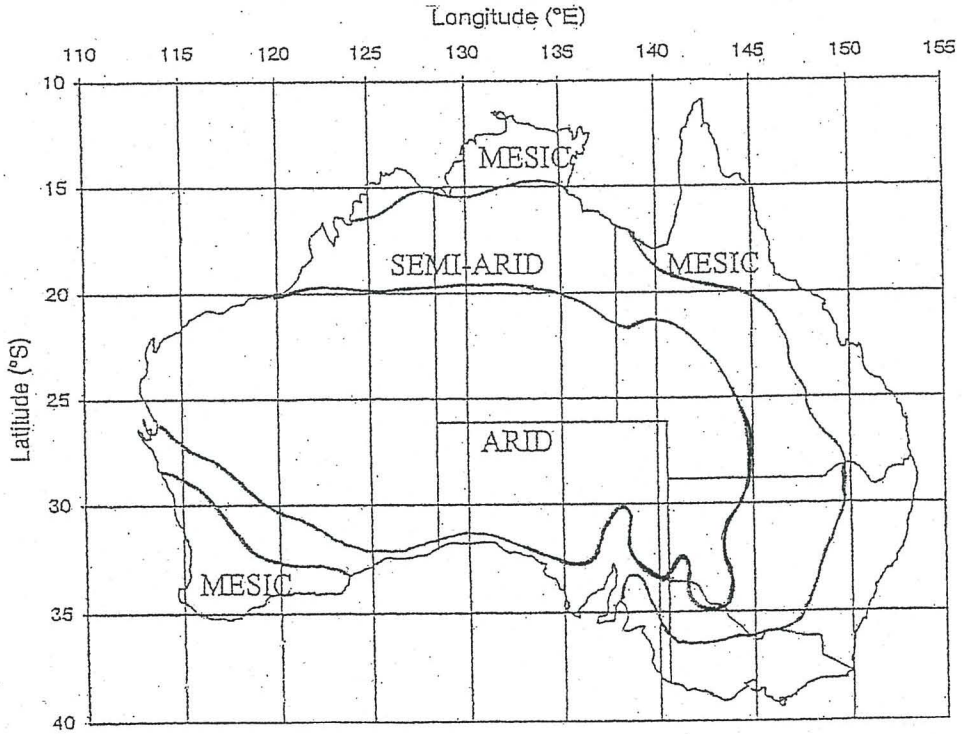

Figure 2.14 Arid, semi-arid and mesic regions of Australia (after Davey, 1983). The inner and outer boundary lines represent approximately 300 mm and 600 mm annual precipitation respectively.

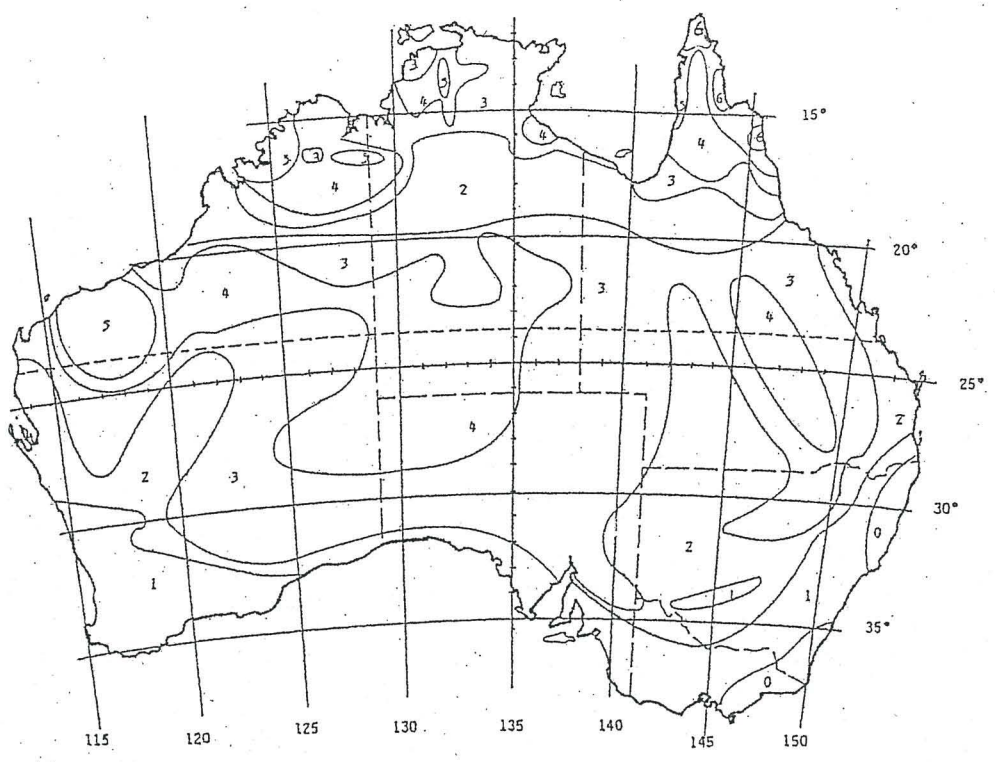

Figure 2.15 Australian gekkonine species densities showing low species densities except in far north.

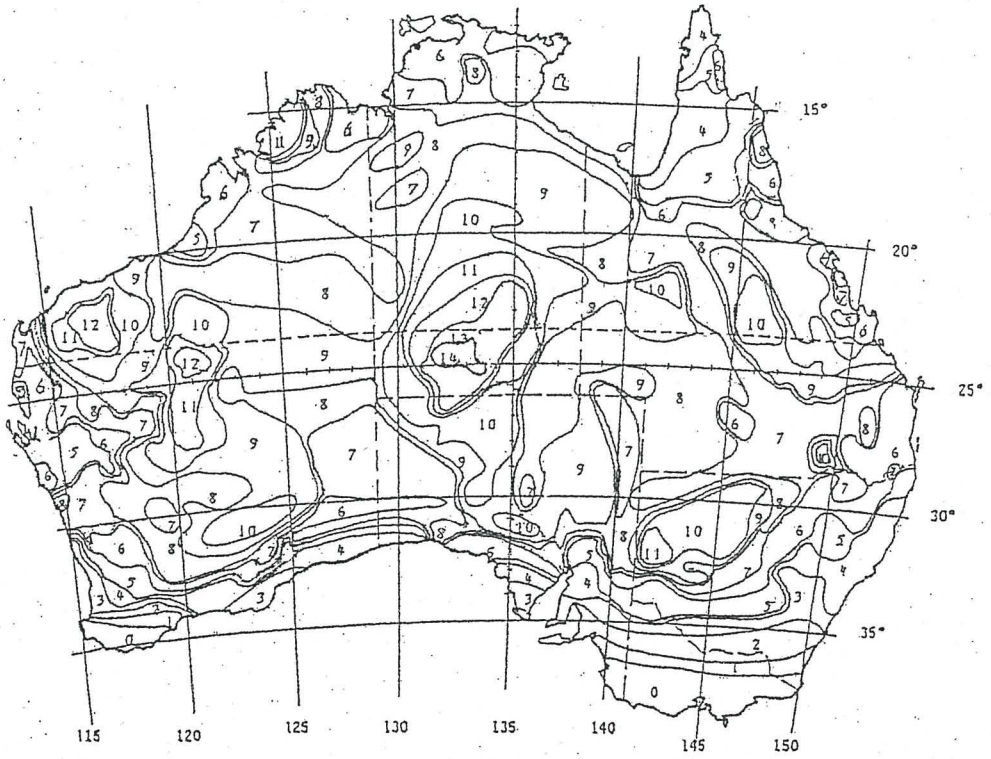

Figure 2.16 Australian diplodactyline species densities showing highest species densities in arid regions.

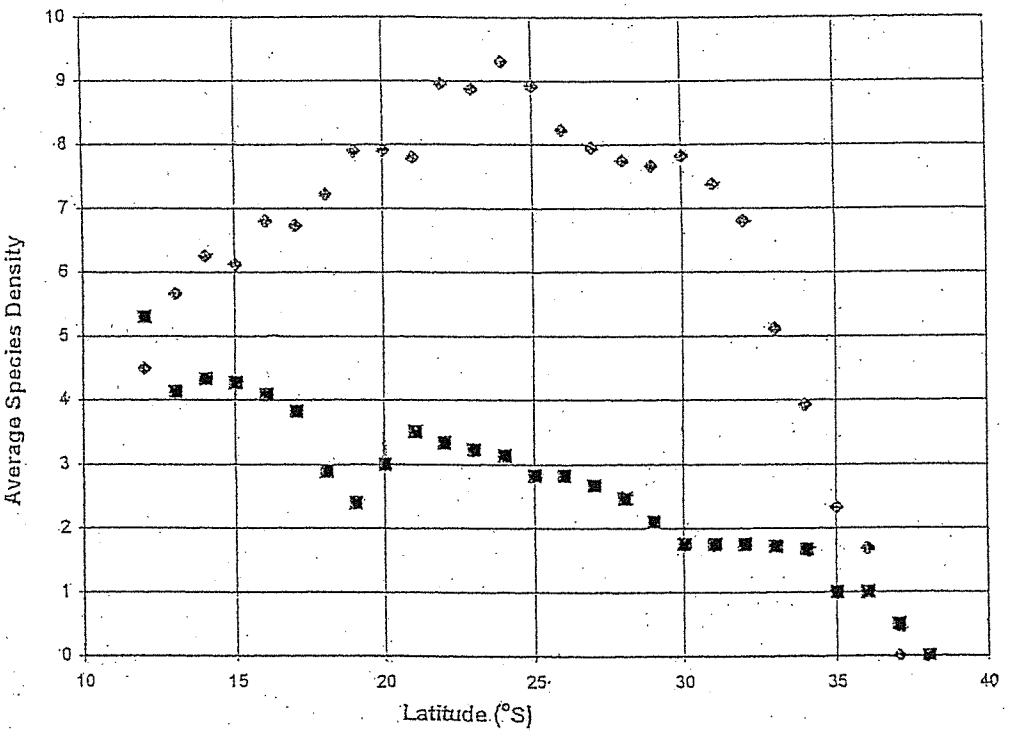

Figure 2.17 Mean species densities of Australian gekkonines (squares) and diplodactylines (diamonds) with latitude, based on 619 samples.

The finding that most *Nephrurus* and *Underwoodisaurus* species distributions are extended latitudinally more than longitudinally suggests that temperature may be a more significant factor than aridity in limiting dispersal and speciation. Two of the four species with longitudinally greater than latitudinal distributions (*N. asper*, *N. wheeleri*) have largely tropical distributions, where there is less variation in temperature with latitude, and the other two species (*N. deleani and U. sphyrurus*) have relatively small habitat-restricted distributions.

Species distributions that range between tropical and temperate or arid and mesic suggest varied life history patterns in differing localities. The fact that six gekkonine species have adapted to largely arid regions demonstrates a significant degree of life history plasticity, (a phenomenon that has also occurred on other continents (Pianka, 1986)).

The reasons for the rapid southerly decline to a mean of about 2.5 gekkonine species at about 20 °S right across Australia (Figures 2.15, 2.17) are not known, but it occurs in a tropical region of high diplodactyline species density that has been poorly collected. Also the dip is situated in the semi-arid region between the monsoonal zone of the north and the central arid zone, and experiences increased stress of both temperature and rainfall extremes. The coincidence of species density peaks for both gekkonine and diplodactyline species at about 24-25°S suggests a common factor; one such factor is aridity, but others, such as reduced predation pressure could be advanced. Current distributions are related to palaeoclimatic factors as well as times of arrival in Australia (Cogger and Heatwole, 1981). The dominance of the Diplodactylinae in central Australia is probably largely due to the longer period of adaptation compared to the Gekkoninae, which has its highest species densities in coastal areas, indicating multiple, geologically recent transoceanic arrivals.

The Australian mainland Diplodactylinae (80 species) (Figure 2.16) show a significantly more complex distribution pattern than that for the Gekkoninae, no doubt reflecting the greater species diversity. The low (inverse) correlation of species density and latitude across Australia is confounded by the shape of the species density curve (Figure 2.17). The strong inverse correlation of species density with latitude south of the Tropic of Capricorn is expected, but why diplodactyline numbers (but not gekkonine) are reduced to zero in the extreme south-west and parts of south-east of Australia is not clear. The highest species density of 14 in central Australia, and most of the other areas of high species density, occur in areas of high aridity. The overall tendency indicates that geckos of the Diplodactylinae have speciated more in the arid and warm temperate zones. The success in arid zones where annual precipitation may be less than 100 mm (Gentilli, 1978) is despite the fact that diplodactyline eggs are all parchment type and therefore desiccation intolerant.

Among the diplodactylines the trend of species density with latitude, consists of three parts (Figure 2.17). The initial section from 11 - 24°S (Tropic of Capricorn) shows increasing species density with increasing aridity within the tropical zone. In this same region there is a decreasing gekkonine species density, but it is not possible to say whether this relationship is competition based, because factors such as biomass density and effect of other insectivorous species are unknown. The second section from about 24 - 30 °S shows slowly reducing average species densities as average temperatures decrease. The third

section of the graph shows a more rapid and consistent decline in average species densities reaching zero at 37 °S. Why there is such a strong demarcation between the second and third components is not clear, but decreasing temperatures are a major factor in the decline of species density with increasing latitudes in the temperate zone.

Gecko reproductive strategies that might assist in the colonisation of the coldest regions of Australia have not extended to viviparity, as has happened in New Zealand geckos (Gill and Whitaker, 1996) and also in one New Caledonian gecko (Bartmann and Minuth, 1979). Freshly laid (South African) gecko eggs with moderately advanced embryos have been recorded (Power, 1939) and, although egg retention has been observed in *Nephrurus* and *Underwoodisaurus* (Harvey, 1983; Chapter 5), this adaptation has never led to the evolution of viviparity in any Australian geckos. The fact that viviparity has not occurred in any eublepharine, sphaerodactyline or gekkonine gecko species suggests that such an evolutionary step is major and that it is accompanied by costs which are difficult to accommodate. The reason why there are no viviparous geckos in Australia may be partly because geckos are believed to have radiated from the warm north to the cool south (Cogger, 1984), and have never reached the cold climate regions of Tasmania. The climate around Canberra and Cooma, where *Diplodactylus vittatus* and *Christinus marmoratus* occur at elevations up to 1000 m, includes a relatively prolonged cold winter and relatively short mild summer. Such conditions must be close to the limits for sustainable reproduction in these species. At these higher altitudes, reproduction cannot occur more than once per season, and, as in certain other high altitude lizards, possibly not every year (Edwards et al., 2002). Any selection towards viviparity among lizards is countered by the fact that viviparity is energetically more costly than oviparity (Pianka, 1986). Evidently the selection pressures are not sufficiently strong in the adjacent higher and cooler regions for the development of viviparity in Australian geckos.

The larger proportion of arid diplodactylines compared to arid gekkonines can be explained on the basis that the gekkonines are presumed to have had less time to adapt than the diplodactyline geckos. An alternative possibility is that there is a fundamental difference in the reproductive biology of the two groups (Chapters 4, 5). It is also possible that both adaptive and phylogenetic characteristics contribute to the distributional differences. With the current limited knowledge of physiology, distributions and microhabitats it is not

possible to say what interactions occur between gecko species or whether for example any gekkonines have displaced diplodactylines. The fact that two endemic Australian gekkonine genera (*Christinus* and *Heteronotia*) have widespread distributions and, in the case of *Christinus*, has reached further south than the diplodactylines, suggests that these lineages may have been in Australia longer than other gekkonines. These distributions also suggest that there is no phylogenetic constraint for the southerly progression of gekkonines across arid Australia.

Chapter 3

Morphometric and Meristic Analyses of the Genera *Nephrurus & Underwoodisaurus*

3.1 Introduction

3.1.1 Sexual Dimorphisms

A sexual dimorphism (SD) may be defined as any consistent phenotypic difference between males and females of a species (Andersson, 1994). One common dimorphism found in many animal species, including geckos, is that of sexual size dimorphism (SSD). Three major hypotheses are used to explain SSD in reptiles, the competition-avoidance hypothesis, the intrasexual selection hypothesis (Schoener 1977; Stamps, 1983) and the larger offspring hypothesis (Shine, 1978a, 1985b). The competition-avoidance hypothesis states that size dimorphism reduces competition between males and females *e.g.*, for the same sized prey items (Simbotwe, 1985; Bourne et al., 1986; Heatwole and Taylor, 1987). The intrasexual selection hypothesis, states that mates are chosen in a manner that enhances the evolutionary success of the species *e.g.*, choosing a larger female mate may result in more offspring (Tinkle et al., 1970; Schoener, 1977; Stamps, 1983; Carothers, 1984). In the larger offspring hypothesis, larger females can produce larger offspring that are better able to survive. In each case any SD has a corresponding functional significance. I predict that the identification of additional dimorphisms can be used to better understand the characteristics of the reproductive strategies of geckos of the genera *Nephrurus* and *Underwoodisaurus*.

Numerous morphological SDs occur in reptiles, but not all occur in geckos (Stamps, 1983; Obst et al., 1988; Shine, 1990). Sexual dimorphisms of colour, SVL, head size, limb length, toe length, cloacal bones, cloacal sacs, endolymphatic sacs, and preanal pores have been described in Eurasian and African geckos (Loveridge, 1947; Beutler, 1981; Werner and Sivan, 1993). The SDs are so slight in some species that even adult specimens are difficult

to sex *e.g.*, *Teratoscincus* (Miller, 1984). Structure-function relationships are poorly understood for most SDs in *Nephrurus* and *Underwoodisaurus e.g.*, cloacal spurs and body size difference. In *Nephrurus* and *Underwoodisaurus* only the internal reproductive organs, body size (How et al., 1990) and postcloacal tubercles (Rieppel, 1976; Russell, 1977; Kluge, 1982; Bastinck, 1986) have been reported to be sexually dimorphic.

3.1.2 Gecko Morphology

The major aim of this component of the study was to investigate the morphology of *Nephrurus* and *Underwoodisaurus* in order to investigate their systematic relationships but with an emphasis on reproductive structure/function relationships. The morphometric and meristic measurements are mainly those made in standard taxonomic and reproductive works on lizards, and include head, body, tail and limb measurements, as well as scale counts and measurements (Appendix 1). Gonad measurements and general aspects relating to reproductive biology are presented in Chapter 4, and aspects relating to eggs are covered in Chapter 5. Morphometric and meristic analysis is important in determining the identity of the species being studied, providing support for phylogenetic relationships, providing evidence of life-cycle strategies and also, for providing the determination of characteristics that might give a selective advantage to life in arid environments.

Water loss can be a major problem for arid region reptiles (Minnich, 1982). Epidermal keratin and lipids provide some protection against aridity (Zucker and Maderson, 1980; Lillywhite and Maderson, 1982), thus a more keratinised epidermis might be expected in arid adapted species. However, this is not always the case, particularly among nocturnal geckos which can hide in relatively cool, moist burrows during the day and emerge at night when the vapour pressure deficit is much less (Heatwole and Taylor, 1987). The females, at least, of diplodactyline geckos, must be able to dig moderately deep burrows for oviposition in arid regions (Wagner and Lazik, 1996). This implies that efficient digging capacity might be revealed in the functional morphology of limbs and digits. Arid environments will act equally on both males and females (unless there are significant differences in behavioural patterns); thus it would be expected that, in general, morphological characters related to aridity would not show SDs.

3.2 Methods

I examined and measured 1208 *Nephrurus* and *Underwoodisaurus* specimens (Appendix 1). The majority of observations were made on spirit specimens from all the major Australian State and Territory Museums, and included specimens of all eleven species. Most available specimens were examined except, in the case of the three species with relatively larger numbers (*N. laevissimus, N. levis and U. milii*), where random subsamples were examined. Many live and preserved specimens were also photographed or photocopied to record morphological characteristics. Measurements from various opportunistically acquired or museum specimens of out-group species (*Phyllurus, Diplodactylus* and *Oedura* species) and some geckos of other genera (including gekkonines) were also made during the course of this study to help determine whether characteristics were likely to be phylogenetically or adaptively determined.

Field observations were made in Western Australia, South Australia, New South Wales and Queensland. Live geckos were collected by hand from beneath rocks, logs and surface debris by day, and also by spotlighting at night. Live observations were made on captive specimens of nine of the eleven *Nephrurus* and *Underwoodisaurus* species (*N. amyae, N. asper, N. deleani, N. laevissimus, N. levis, N. stellatus, N. wheeleri, U. milii and U. sphyrurus*). Suitable husbandry conditions were developed to promote reproduction in captivity (Appendix 3). Males of each species were placed together briefly in pairs to determine whether they were territorial. In addition to commonly used measurements and observations, a number of characters that general and behavioural observations suggested might be linked to reproduction were also included *e.g.*, pelvic width, rostral scale dimensions and caudal knob characters (Appendix 1).

All linear measurements are in mm and volume measurements in mm^3 (or μL), unless indicated otherwise. The majority of measurements were made once using a digital micrometer calliper accurate to 0.01 mm and with modified jaws to allow measurement of internal structures with a minimal incision. Unusual or outlier measurements were double-checked. Most of the smaller measurements were made under an Olympus dissection microscope at x20 or x40 magnification. Some of the high magnification (*e.g.*, cytological) observations were made using an eyepiece micrometer or eyepiece calibration grid. Most

meristic characters were determined by microscopic examination using an Olympus stereomicroscope. Radiography was carried out on samples of all *Nephrurus* and *Underwoodisaurus* species, including some live specimens, to assist with the analysis of skeletal structures. X-rays were taken of preserved specimens using a fine grain Industrex X-ray film with exposures of 30 s at 30 kV and a focus-film distance of 1 m using a Seifert, Eresco 150/5 BE machine. Live specimens were exposed for 0.5-1 s at 60 kV.

I averaged the measurements for all sexually mature specimens for comparison of snout-vent lengths in males and females; log transformed the data, and then performed a t-Test on the means. Gender was determined in live adult and most juvenile specimens on the basis of the presence (in males) of the hemipenial bulges or their absence (in females). A few hatchlings could not be gender-assigned until they were a month or more old. More than 99% of all specimens examined were reliably sexed by this method. Dissection and exposure of the gonads (of preserved specimens) was necessary for sex determination of a few juveniles and hatchlings. Dissected specimens, including recent hatchlings and advanced embryos, were sexed by examination of the gonads under x40 magnification. The ovaries of very young females were distinguished from testes by the presence of very small but distinctive blister-like primary follicles (Chapter 4). Sex ratios were analysed using the Chi-squared test for each species, for each genus, and for *Nephrurus* and *Underwoodisaurus* combined.

A relative size was used for comparison of morphometric data between sexes and among individuals and species because each species has a different mean size and many of the specimens examined were at different stages of growth. Relative dimensions refer to the size relative to the SVL of the animal (*e.g.*, Werner, 1969). Sexual size dimorphisms were determined by comparing the means of SVL of the ten largest adult specimens of each gender using a t-Test where $P = <0.05$. For species with inadequate numbers, smaller equal samples were used. For percentage and other ratios, the values were arcsin transformed prior to testing (Sokal and Rohlf, 1995). Trends were confirmed using linear regression analysis and reduced major axis analysis (Seim and Saether, 1983). Possible bias in sex ratios was tested using the Chi squared test.

3.3 Results

The list of specimens examined is given in Appendix 2 and the character measurements, observations and certain derivatives for males and females of each *Nephrurus* and *Underwoodisaurus* species are given in Appendix 1, (Tables A1.1-A1.11). For comparison the measurements of some diplodactyline out-group species are given in Table A1.12.

3.3.1 Sex Ratios of *Nephrurus* and *Underwoodisaurus* Geckos

There was no significant sex ratio bias in any species of *Nephrurus* or *Underwoodisaurus* in the samples I examined (Table 3.1), using the Chi-squared test, except for *N. vertebralis* where P = 0.093 (excess males).

3.3.2 Sex Dimorphisms

There are 30 external characters that show a SD where P = <0.05. In addition, a distinct escutcheon of enlarged, mid-ventral scales, adjacent and posterior to the umbilical scar is only found in adult males of *U. milii* (Figure 3.1) and *U. sphyrurus*. The escutcheon referred to here has not been examined histologically but is probably not homologous to the exocrine escutcheon described in certain sphaerodactyline genera (Maderson, 1972). A possibly vestigial escutcheon consisting of a patch of slightly enlarged midventral scales is found in some *N. asper* group and *N. stellatus* males and females, also in adult female *U. milii*. In addition, well-differentiated preano-inguinofemoral tubercles (Figure 3.2) are only found in adult males of *N. deleani, N. laevissimus, N. levis* and *N. stellatus*. Well-developed cloacal tubercles of various forms are found in adult males of all *Nephrurus* and *Underwoodisaurus* species (Figure 3.3).

The most significant of the dimorphisms are analysed in more detail below (Tables 3.2 - 3.5). Adult females are significantly larger than adult males in all *Nephrurus* and *Underwoodisaurus* species except *U. milii* (Table 3.2). Only one species (*Phyllurus* platurus) out of six diplodactyline out-group species showed a SSD (Table 3.2, Appendix 1, Table A1.12). Adult males have relatively longer tails than adult females in all species except *U. sphyrurus* (Table 3.2, Figures 3.4, 3.5). All species have a greater relative head width in males than females, except *N. amyae, N. deleani,*

N, stellatus and *N. vertebralis* (Table 3.3, Figure 3.6). *Nephrurus amyae*, *N. laevissimus, N. levis, N. sheai* and *U. milii* have relatively longer heads in males compared to females (Table 3.3). The cloacal spurs are more pronounced in adult males than females, so the relative transtubercular width is greater in the adult males of all species except *N. amyae* (Table 3.4, Figure 3.7). The relative transiliac width is significantly greater in adult females of *N. amyae, N. laevissimus, N. vertebralis* and *U. milii* than in males (Table 3.4, Figure 3.8).

Table 3.1 Sex ratio of *Nephrurus* and *Underwoodisaurus* species (N = number of specimens, P = χ^2 probability value).

SPECIES	MALES (N)	FEMALES (N)	MALES:FEMALES	P
N. amyae	9	11	0.81:1	0.655
N. asper	38	35	1.09:1	0.725
N. deleani	9	12	0.75:1	0.513
N. laevissimus	137	128	1.07:1	0.580
N. levis	96	80	1.20:1	0.228
N. sheai	12	21	0.57:1	0.117
N. stellatus	19	15	1.27:1	0.493
N. vertebralis	34	15	2.26:1	0.093
N. wheeleri	17	18	0.94:1	0.866
Totals (*Nephrurus*)	**371**	**334**	**1.11:1**	**0.087**
U. milii	139	119	1.17:1	0.213
U. sphyrurus	13	15	0.87:1	0.705
Totals (*Underwoodisaurus*)	**152**	**134**	**1.13:1**	**0.193**
TOTALS (all 11 species)	**523**	**468**	**1.12:1**	**0.161**

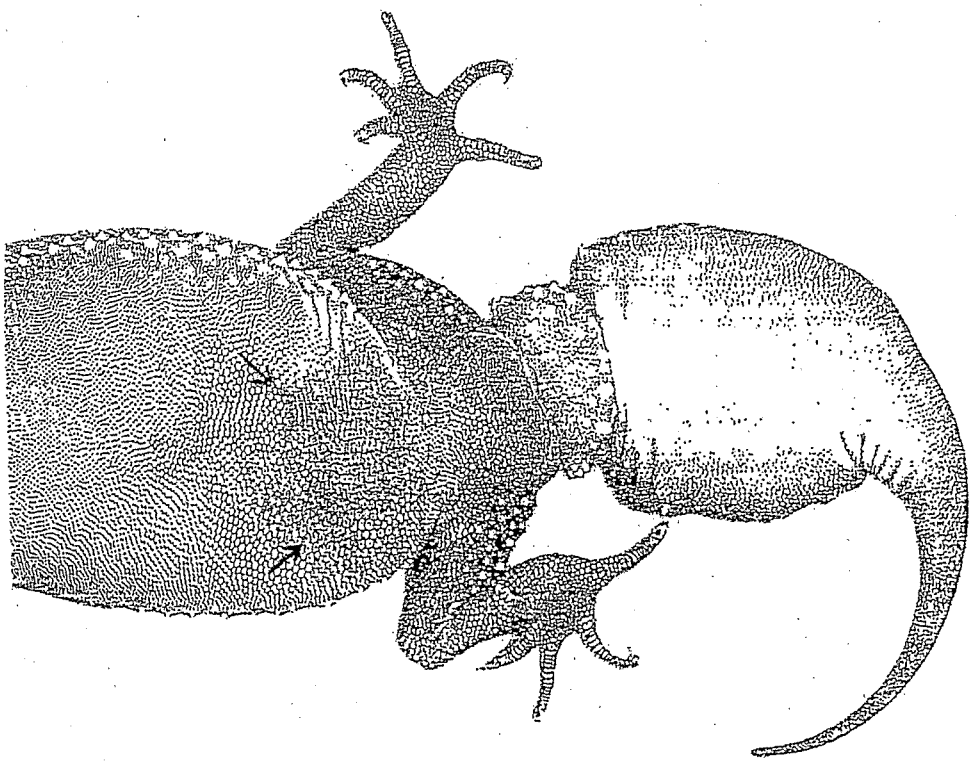

Figure 3.1 Escutcheon (x2) of enlarged flattened and slightly translucent scales found in adult male *U. milii* (arrows), during the breeding season.

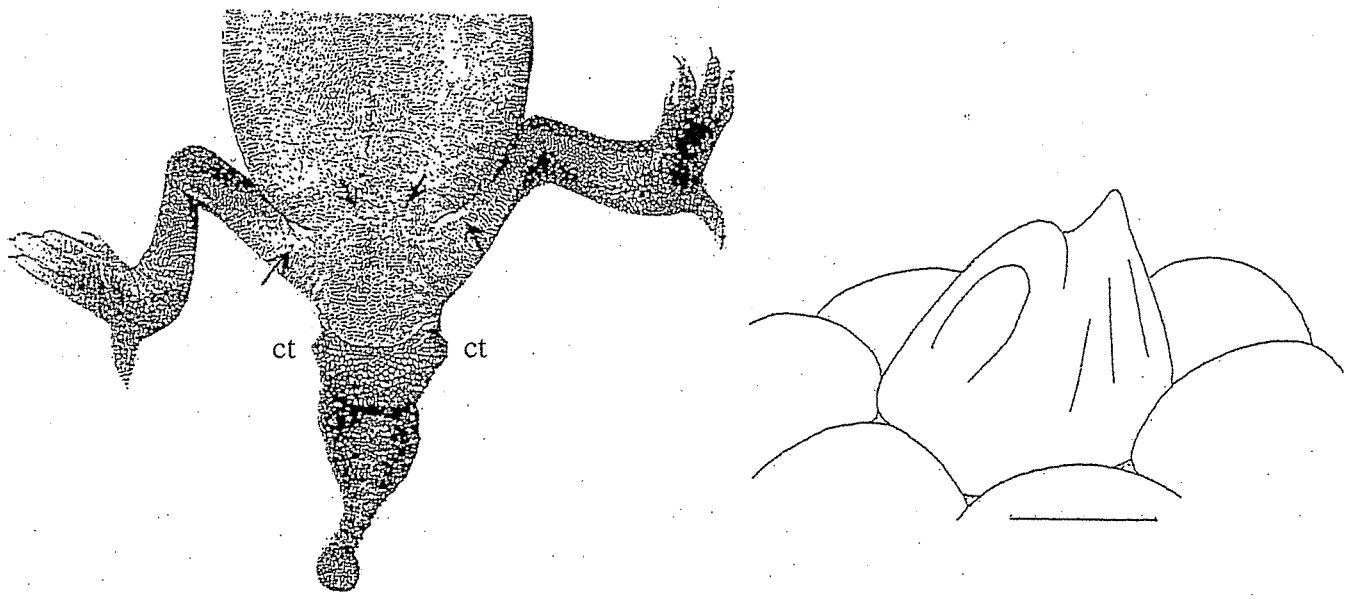

Figure 3.2 Ventral surface (x2) of an adult male *N. deleani* (left) to show the enlarged, specialised preano-inguinofemoral tubercles (dark spots, arrows), cloacal tubercles (ct) and (right) the anterolateral aspect of a tubercle, scale bar = 1.0 mm.

Table 3.2 Maximum adult SVL and mean adult tail length in *Nephrurus*, *Underwoodisaurus* and six outgroup species (F = female, M = male, N.S. = not significant, N.A. = not available, N = number of adults measured).

SPECIES	MAXIMUM SVL (mm) (F/M%)	T-TEST	MEAN TAIL LENGTH AS %SVL (M/F)	T-TEST
N. amyae	137/110 (125%)	T(2)17 = -4.22, P = <0.001	19.1/18.7	N.S.
N. asper	118/104 (113%)	T(2)44 = -4.35, P = <0.001	22.5/18.9	T(2)46 = 5.90, P = <0.001
N. deleani	89/67 (133%)	T(2)13 = -3.12, P = 0.004	38.0/36.3	T(2)12 = 0.824, P = 0.213
N. laevissimus	95/81 (117%)	T(2)185 = -10.3, P = <0.001	32.7/31.8	T(2)146 = 1.89, P = 0.030
N. levis	98/81 (121%)	T(2)127 = -7.94, P = <0.001	43.7/41.0	T(2)89 = 3.64, P = <0.001
N. sheai	121/100 (121%)	T(2)29 = -2.93, P = 0.003	19.1/15.9	T(2)27 = 2.61, P = 0.007
N. stellatus	86/78 (110%)	T(2)25 = -2.46, P = 0.011	33.7/32.1	N.S.
N. vertebralis	90/78 (115%)	T(2)38 = -3.53, P = <0.001	37.7/36.8	N.S.
N. wheeleri	103/83 (124 %)	T(2)23 = -2.14, P = 0.022	40.6/39.0	N.S.
U. milii	110/95 (116 %)	N.S. (N = 200)	73.5/67.1	T(2)62 = 3.53, P = <0.001
U. sphyrurus	85/73 (116 %)	T(2)35 = -3.12, P = 0.002	52.0/51.6	N.S.
Diplodactylus intermedius	70/70.5 (99%)	N.S. (N = 18).	N.A	
Diplodactylus vittatus	61/55 (111%)	N.S. (N = 11)	N.A.	
Phyllurus platurus	98/88 (111%)	T(2)15 = -4.17, P = 0.001	N.A.	
Oedura lesueurii	69/67 (103%)	N.S. (N = 10)	N.A.	
Oedura robusta	88/80 (110%)	N.S. (N = 10)	N.A.	
Oedura tryoni	89/86 (103%)	N.S. (N = 16)	N.A.	

Table 3.3 Mean head width and head length in adult *Nephrurus* and *Underwoodisaurus* species (M = male, F = female, N.S. = not significant).

SPECIES	HEAD WIDTH AS %SVL (M/F)	T-TEST	HEAD LENGTH AS %SVL (M/F)	T-TEST
N. amyae	26.2/27.0	N.S.	28.8/26.7	T(2)8 = 4.52, P = 0.001
N. asper	29.0/26.7	T(2)48 = 4.54, P = <0.001	29.0/28.6	N.S.
N. deleani	25.4/24.0	N.S.	29.6/28.4	N.S.
N. laevissimus	25.1/24.5	T(2)170 = 4.20, P = <0.001	29.6/28.4	T(2)14 = 2.13, P = 0.026
N. levis	25.9/25.3	T(2)120= 2.11, P = 0.018	28.8/27.6	T(2)21 = 2.42, P = 0.012
N. sheai	27.6/26.6	T(2)23 = 2.12, P = 0.023	28.7/27.2	T(2)21 = 3.60, P = <0.001
N. stellatus	26.6/26.2	N.S.	30.3/30.6	N.S.
N. vertebralis	23.8/23.9	N.S.	28.3/27.5	N.S.
N. wheeleri	26.2/25.1	T(2)28 = 2.50, P = 0.009	27.1/26.3	N.S.
U. milii	22.5/21.3	T(2)146= 5.44, P = <0.001	27.3/25.3	T(2)19 = 5.10, P = <0.001
U. sphyrurus	21.6/21.1	T(2)15 = 2.08, P = 0.027	27.2/25.9	N.S.

Table 3.4 Mean transtubercular and transiliac widths in adult *Nephrurus* and *Underwoodisaurus* species (F = female, M = male, N.S. = not significant).

SPECIES	TRANSILIAC WIDTH AS % SVL(M/F)	T-TEST	TRANS-TUBERCULAR WIDTH AS % SVL (M/F)	T-TEST
N. amyae	11.5/12.4	T(2)8 = -2.94, P = 0.009	11.2/11.1	N.S.
N. asper	12.0/12.3	N.S.	13.1/9.9	T(2)27 = 5.53, P = <0.001
N. deleani	10.8/11.8	N.S.	12.8/8.59	T(2)10 = 3.98, P = 0. 001
N. laevissimus	12.3/12.6	T(2)139 = -3.16, P = <0.001	14.0/10.5	T(2)177 = 16.9, P = <0.001
N. levis	11.9/11.8	N.S.	15.1/10.9	T(2)53 = 8.02, P = <0.001
N. sheai	12.0/12.5	N.S.	12.2/9.3	T(2)22= 3.54, P = <0.001
N. stellatus	13.1/13.4	N.S.	12.3/10.3	T(2)25 = 2.86, P = 0.004
N. vertebralis	10.5/11.1	T(2)31 = -2.05, P = 0.04	12.3/10.2	T(2)33 = 2.65, P = 0.006
N. wheeleri	11.4/11.6	N.S.	17.6/16.4	T(2)19 = 3.89, P = <0.001
U. milii	7.84/9.04	T(2)19 = -2.58, P = 0.009	10.6/9.65	T(2)69 = 2.68, P = 0.004
U. sphyrurus	10.5/10.9	N.S.	19.2/19.1	T(2)21 = 4.54, P = <0.001

Table 3.5 Adult hind-limb length, caudal knob size (*Nephrurus*) and subdigital lamellae numbers (*Underwoodisaurus*), (M = male, F = female, N.S. = not significant).

SPECIES	MEAN HIND-LIMB LENGTH AS % SVL (M/F)	T-TEST	KNOB SIZE** (M/F) (*NEPHRURUS*)	LAMELLAE *** (M/F) (*UNDERWOODISAURUS*)	T-TEST
N. amyae	48.7/46.6	T(2)10 = 2.28, P = 0.023	2.87/2.69		T(2)11 = 1.09, P = 0.101
N. asper	51.4/45.3	T(2)17 = 3.53, P = 0.001	3.57/2.93		T(2)27 = 3.41, P = 0.001
N. deleani	48.4/45.2	N.S.	2.87/2.58		T(2)9 = 1.38, P = 0.101
N. laevissimus	47.1/44.8	T(2)13 = 1.86, P = 0.016	2.78/2.64		T(2)142 = 3.13, P = 0.001
N. levis	46.0/44.3	N.S.	2.57/2.37		T(2)84 = 2.45, P = 0.008
N. sheai	49.2/46.6	T(2)22 = 4.111, P = <0.001	13.36/2.84		T(2)19 = 4.42, P = <0.001
N. stellatus	48.1/46.3	N.S.	3.64/3.82		N.S.
N. vertebralis	49.0/46.6	T(2)31 = 2.34, P = 0.012	2.60/2.44		N.S.
N. wheeleri	48.1/43.2	T(2)23 = 4.65, P = <0.001	2.59/2.34		N.S.
U. milii	45.9/41.5	T(2)20 = 3.99, P = <0.001	85.9/82.3		T(2)23 = 2.23, P = 0.050
U. sphyrurus	50.2/48.0	N.S.	78.9/71.8		T(2)16 = 3.46, P = 0.002

** Knob size is the mean caudal knob dimension (length +breadth +depth) relative to SVL (Appendix 1).

*** Lamellae are defined as the sum total of subdigital lamellae of the five hind-limb digits (Appendix 1).

The limbs are long and slender compared to most *Diplodactylus* and *Oedura* species, and the forelimbs are shorter than the hind-limbs in all species (Appendix 1, Tables A1.1-A1.11). Significant relative forelimb length dimorphism is found in adults of three species (*N. amyae*, *N. sheai* and *N. vertebralis*), and significant relative hind-limb length dimorphism in adults of seven species representing all three study groups (*N. amyae*, *N. asper*, *N. laevissimus*, *N. sheai*, *N. vertebralis*, *N. wheeleri* and *U. milii*); male limbs being relatively longer in all cases (Table 3.5). The relative caudal knob size is greater in males than females in all *Nephrurus* species except *N. stellatus, N. vertebralis* and *N. wheeleri* (Table 3.5, Figure 3.9). The males of *U. milii* and *U. sphyrurus* have significantly more subdigital lamellae than do females, at least on some digits (Figure 3.10, Tables 3.5, A3.10, A3.11). There is no SD of colour or postcloacal sacs. Also, there are no preanal pores or epidermal exocrine glands in any *Nephrurus* or *Underwoodisaurus* species.

The distinctive ventral scales found in *Underwoodisaurus* and some *Nephrurus* species are of two distinct types. The first type, the escutcheon scales, are found in the umbilical area (Figure 3.1). The escutcheon in *U. milii* is more pronounced in adults than in juveniles, is more pronounced in males than females, and is more distinct in the breeding season. The escutcheon scales are pale, with few or no chromatophores, approximately isodiametric, enlarged (approximately twice the diameter of surrounding granules), juxtaposed and flattened with a smooth, waxy, translucent central patch, which, in some specimens, is dimpled in the centre. In females the escutcheon scales are smaller, more hemispherical, juxtaposed scales and they lack the waxy centres or dimples of males.

The weakly defined mid-line escutcheon of enlarged scales, which intergrade with surrounding scales, is found in some adult male and female *Nephrurus*. The post-umbilical zone in *N. stellatus* shows moderate development of an escutcheon area in some specimens. This escutcheon consists of a zone (often bilobed in shape) of slightly enlarged scales, which are more pronounced in adult males than females. The escutcheon scales, which number approximately 30, tend to be flatter and smoother and are sometimes more translucent, particularly towards the centre, or highest point, of each scale, than the

surrounding scales. Some specimens have escutcheon-type scales scattered over the surrounding parts of the ventral surface.

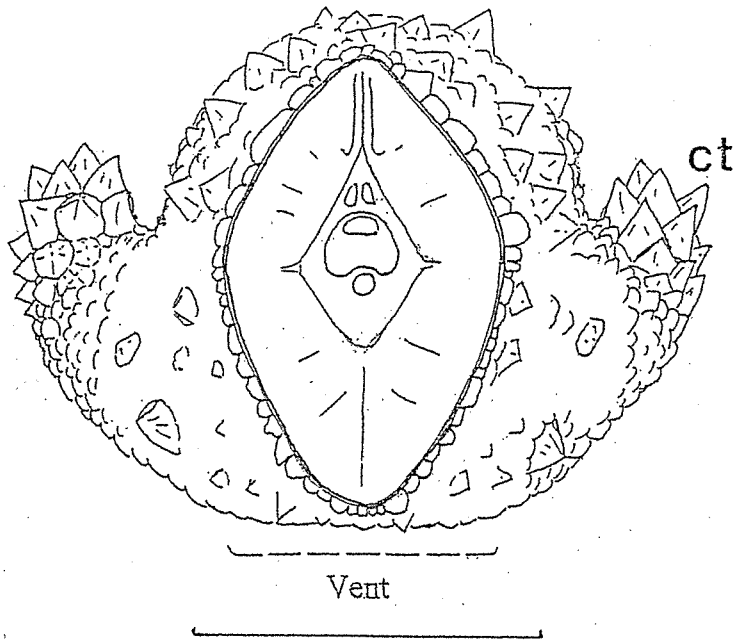

Figure 3.3 Posterior view of pelvic region in adult male *N. levis* with tail removed showing relation of cloacal tubercles (ct) (just posterior to hind legs) and vent, scale bar = 5 mm.

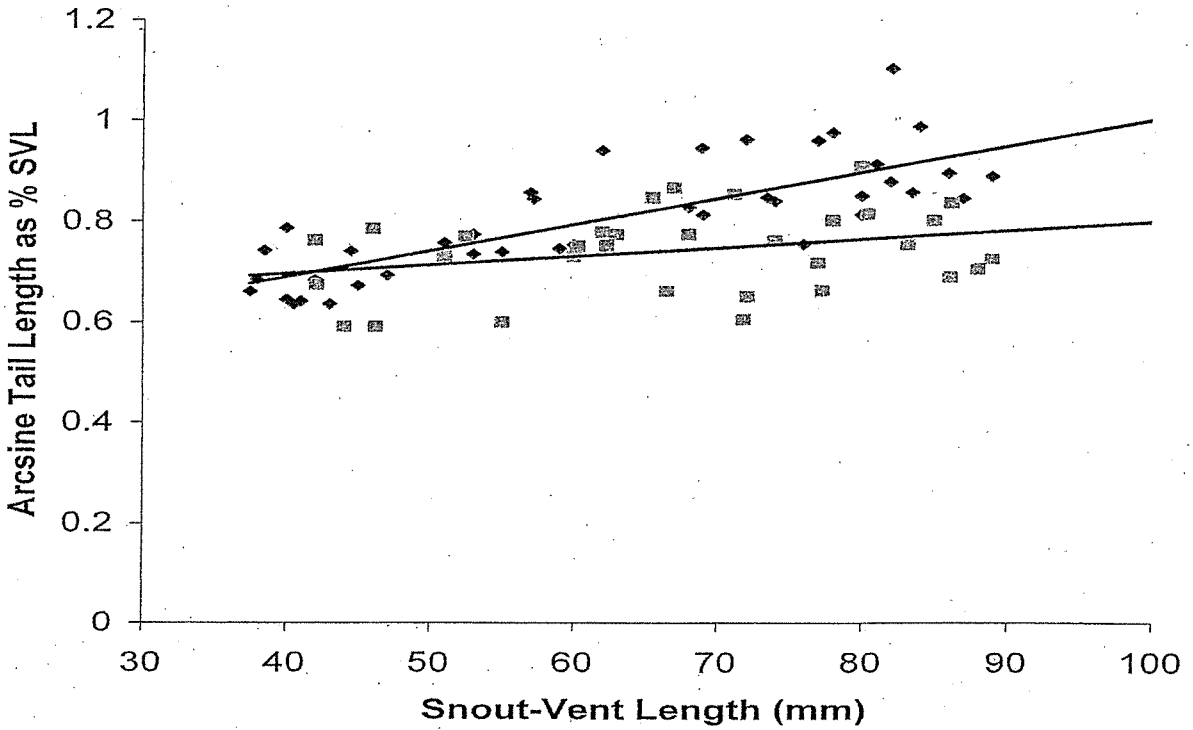

Figure 3.4 Sexual dimorphism in tail length of *U. milii*. The slopes of the linear regressions of the logarithms of tail length on snout-vent length are significantly different for males and females ($t_{(2)132} = 2.51$, $P = 0.0076$), N = 77 males (diamonds, upper line, $R^2 = 0.950$) and N = 57 females (squares, lower line, $R^2 = 0.888$).

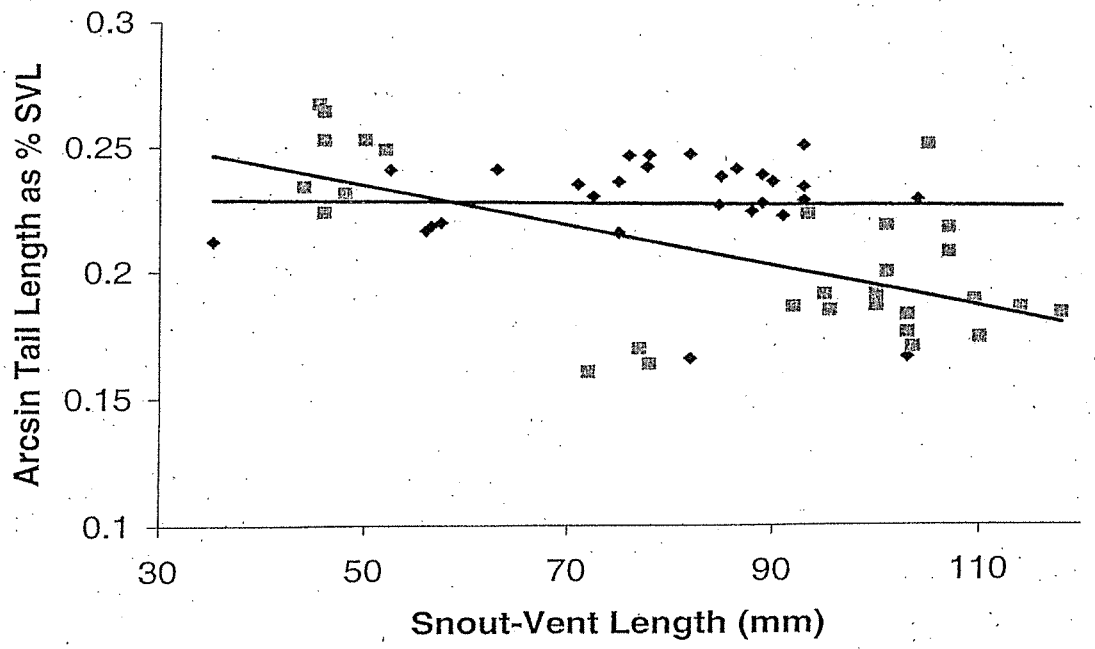

Figure 3.5 Development of tail length in *N. asper*. The slopes of the linear regressions of the logarithms of tail length on snout-vent length are significantly different for males and females ($t_{(2)67} = 3.50$, P = <0.001), N = 37 males (diamonds, upper right line, $R^2 = 0.935$) and N = 34 females (squares, $R^2 = 0.861$).

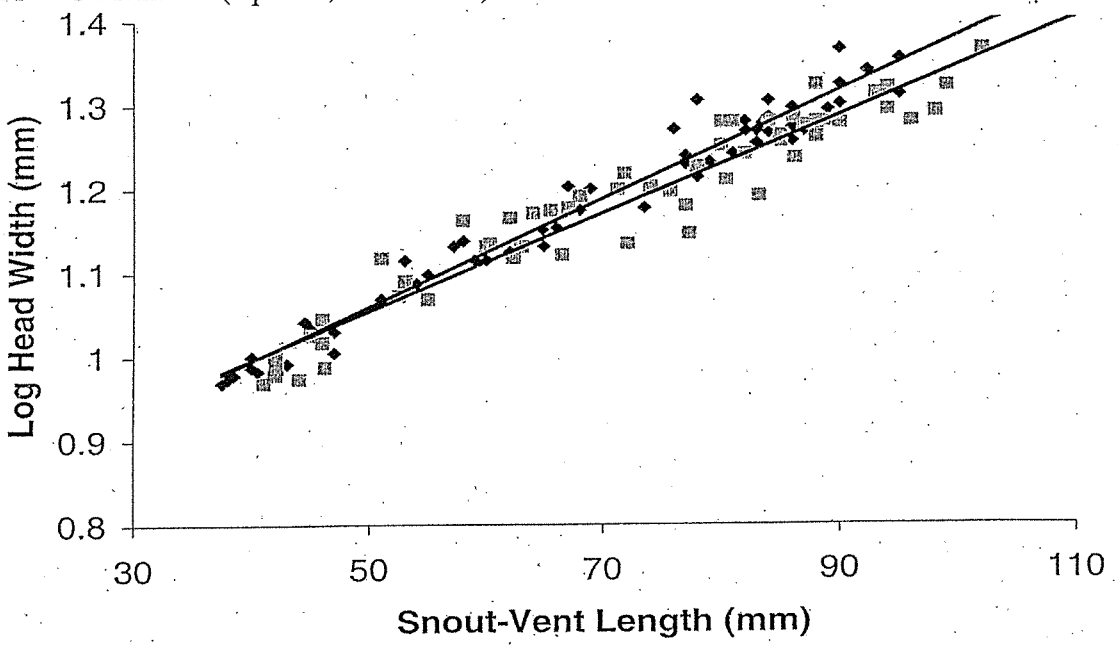

Figure 3.6 Change in relative head width with increase in snout-vent length in *U. milii*. The slopes of the linear regression lines for head width on snout-vent length are significantly different ($t_{(2)243} = 4.14$, P = <0.001) in males (N = 129, diamonds, upper line on right) compared to females (N = 116, squares).

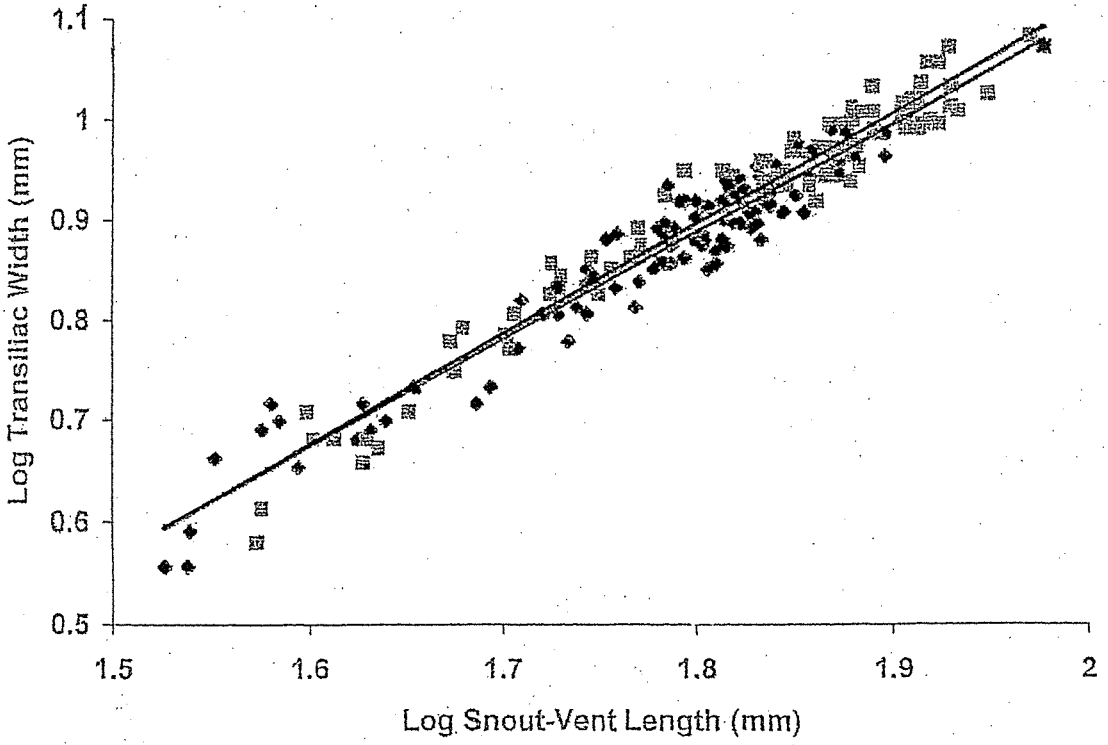

Figure 3.7 Changes in transiliac width during development of *N. laevissimus* males and females. The regression lines of relative transiliac width on snout-vent length are significantly different ($t_{(2)189} = 3.76$, P = <0.001) in males (N = 97, diamonds, lower line) compared to females (N = 101, squares, upper line).

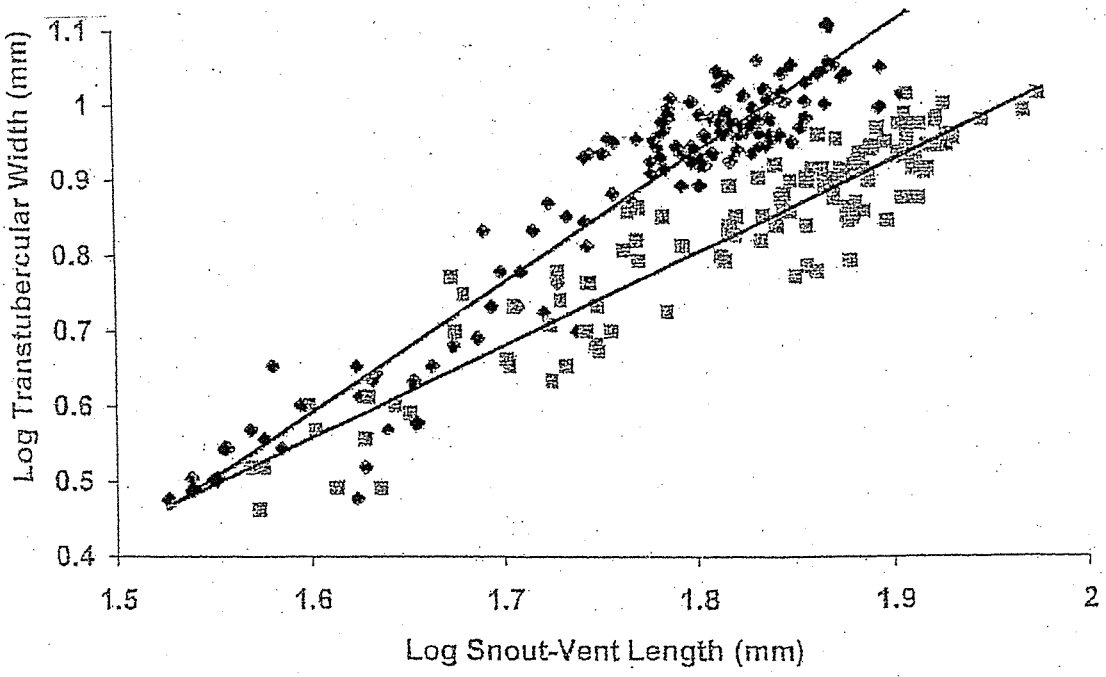

Figure 3.8 Sexual dimorphic development of transtubercular width in *N. laevissimus*. The slopes of the linear regression lines for transtubercular width on snout-vent length are significantly different ($t_{(2)244} = 12.05$, P = <0.001) for males (N = 126, diamonds, upper line) compared to females (N = 120, squares).

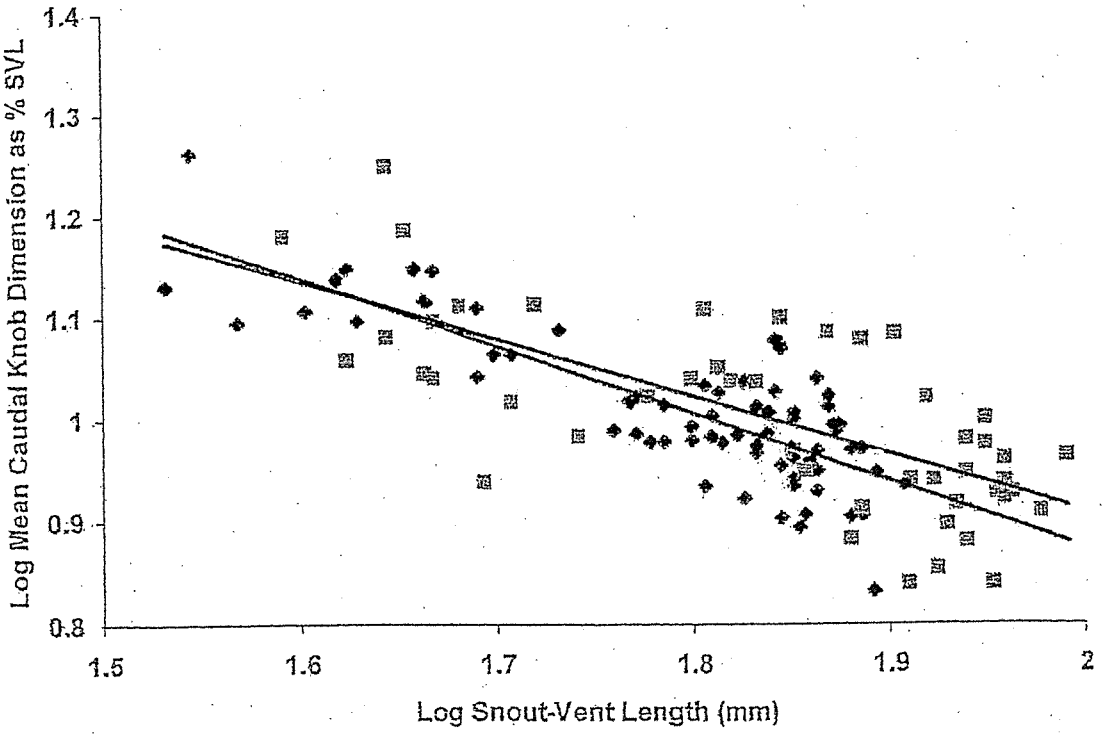

Figure 3.9 Changes in relative size of the caudal knob during adult growth in *N. levis*. The regression lines for the logarithm of mean caudal knob dimension as % snout-vent length on logarithm of snout-vent length are significantly different for males and females ($t_{(2)87}$ = 2.09, P = 0.019). For males N = 70 (diamonds, lower line) and for females N = 48 (squares).

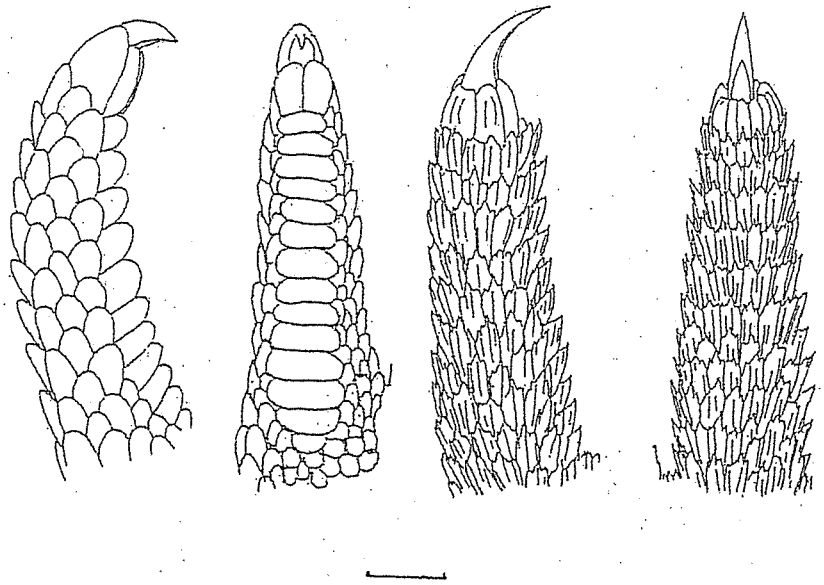

Figure 3.10 Comparison of subdigital scales in *Underwoodisaurus* and *Nephrurus* species, lateral and inferior views of manus digit I from *U. milii* (left) and *N. levis* (right), scale bar = 1 mm.

The second type of specialised ventral scale consists of distinctive conical tubercles that are scattered across the preanal zone, the proximal subfemoral zone and (in some specimens) the inguinal zone, of adult males of *N. deleani*, *N. laevissimus*, *N. levis* and *N. stellatus* (Figure 3.2). For example there is a patch of approximately 50 scattered, enlarged tubercles with a highly modified micromorphology in *N. deleani*. These tubercles extend from the immediate post umbilical area to half way to the vent and also extend laterally to the inguinal region and approximately two thirds of the way along the underside of each thigh (Figure 3.2). They are opaque white, and variable in diameter from approximately the same diameter as the surrounding granules to nearly twice the diameter of these granules (0.13-0.22 mm).

Although there is a range in degree of differentiation, each fully developed specialised tubercle has a short projecting spine towards the rear and a transversely oriented furrow and promontory on the anterior aspect of the tubercle, with a translucent facet on the anterior surface (Figure 3.2). In *N. levis* there are approximately 20-30 similarly distributed tubercles. Some of the tubercles have a pale pink tinge in some live specimens. In *N. stellatus* there are approximately 15-35 similarly distributed tubercles. These tubercles are barely discernible in male hatchlings of *N. levis*.

3.3.3 Distinctive Morphological Characteristics

The *asper* group has the relatively (and absolutely) largest caudal knobs in the genus, the shortest tails and the least potential for tail fat storage (Chapter 6). Among the remaining *Nephrurus* species, *N. deleani and N. laevissimus* also have relatively very small tails (Figures 1.3, 1.4) *whereas N. levis and N. wheeleri* (Figures 1.5, 1.9) have relatively long and broad tails with potential for fat storage. Compared to original tails, the regrown tails of *Nephrurus* and *Underwoodisaurus* spp. are invariably shorter, less colourful, less ornate, lack a bony skeleton but are more bulbous. In *Underwoodisaurus* (unlike *Nephrurus*) species the well-developed regrown tails have a pointed extremity, which however, is less pronounced in *U. sphyrurus*.

Radiography confirmed that the more recently described *N. amyae*, and *N. sheai* both possess the more primitive phalangeal formula, 2.3.4.4.3 /2.3.4.4.4 (as in *N. asper* and *N. wheeleri*). Radiography also confirmed that *N. deleani* has the more derived phalangeal

formula with further loss of phalanges 2.3.3.3.3 /2.3.3.3.3 (as in the *N. levis*, *N. laevissimus*, *N. stellatus* and *N. vertebralis* group).

The nostril does not contact the rostral or labial scales in any *Nephrurus* and *Underwoodisaurus* species. All *Nephrurus* species have multiple small granules between the nostril and the rostral scale, whereas *Underwoodisaurus* species only have a single enlarged scale. Another distinctive characteristic of *Underwoodisaurus* is that the adult males have a mid-ventral patch of enlarged and specialised scales or escutcheon, but with no evidence of exocrine pores. In *Nephrurus* species the escutcheon is either absent, or indistinct with less specialised scales. All *Nephrurus* species have largely irregular mucronate and carinate subdigital scales, compared to smooth, slightly swollen, monoseriate subdigital lamellae in *Underwoodisaurus* species (Figure 3.10). The apical subdigital lamella on all digits in *Underwoodisaurus* species is usually divided symmetrically. The rostral scale is undivided in all *Nephrurus* and *Underwoodisaurus* species (except in *N. wheeleri* where it is frequently divided asymmetrically). The laterally enlarged mental scale contacts numerous small postmental scales in *Nephrurus* species (means range from 12.0 - 15.8 in the more derived *Nephrurus* species group and from 7.4 - 10.2 in the more primitive species group). The mental scale contacts fewer slightly enlarged postmental scales (means range from 3.5 - 5.7) in *Underwoodisaurus* species.

3.3.4 Behavioural Observations

No autotomy was found in *N. amyae*, *N. asper* and *N. sheai*. The tail regrowth rate among the remaining *Nephrurus* species averages 5.1% in males and 6.1% in females (Table 3.6). The comparable rates among *Underwoodisaurus* geckos are 16.3% and 17.1% respectively (Table 3.6). Only one *Nephrurus* (*asper*) specimen had a damaged tail knob (of over 420 *Nephrurus* tails examined), with a small piece sliced out of the topside. The wound had healed leaving a scar without apparent regrowth of the wounded area. A second specimen that had lost the caudal knob had regrown scales over the tip of the peduncle, but there was no regrowth of the knob. One poorly preserved specimen lacked a tail knob, but it may have been lost after collection.

No evidence has been found of eggs being laid in natural crevices and it is presumed that all *Nephrurus* and *Underwoodisaurus* females dig oviposition burrows. In captivity

N. asper, N. deleani, N. levis, N. stellatus and *U. milii* dig refugia. *Nephrurus asper, N. stellatus, U. milii and U. sphyrurus* have all been found in natural crevices under logs and rocks, and *N. levis* has been found in small mammal burrows (Cogger, 2000). A distinctive characteristic of *Nephrurus* species is that they will often block the burrow entrance when re-entering the burrow (Schmida, 1973; Galliford, 1978; Delean, 1982). Frequently there is so much substrate pushed into the entrance from inside that a pile of material outside the entrance may result. Occasionally the blockage is incomplete and a small hole is left at the top of the burrow entrance (Delean, 1982, personal observation). Burrow blocking behaviour has not been observed in *Underwoodisaurus* geckos and no burrowing has been observed in *U. sphyrurus* except by females about to lay eggs.

Table 3.6 *Nephrurus* and *Underwoodisaurus* Geckos with Tail Regrowth

SPECIES	MALES	N	RATE %	FEMALES	N	RATE %
N. amyae	0	9	0	0	11	0
N. asper	0	29	0	0	27	0
N. deleani	0	8	0	0	12	0
N. laevissimus	3	130	2.3	8	127	6.3
N. levis	9	94	9.6	6	79	7.6
N. sheai	0	11	0	0	21	0
N. stellatus	1	19	5.3	0	15	0
N. vertebralis	1	27	3.7	2	13	15.4
N. wheeleri	1	14	7.1	0	17	0
TOTALS*	**15**	**292**	**5.1**	**16**	**263**	**6.1**
U. milii	11	67	16.4	10	63	15.9
U. sphyrurus	2	13	15.4	3	13	23.1
TOTALS	**13**	**80**	**16.3**	**13**	**76**	**17.1**

* Totals exclude *asper* group.

N.B. All species show no significant difference between male and female tail autotomy/regrowth but *Underwoodisaurus* species show more than *Nephrurus*.

Both sexes of *N. amyae, N. asper, N. levis* and *N. stellatus* will often press the caudal knob into the substrate and swish it from side to side in a tight curve when about to pounce on prey. *Nephrurus levis* will often press the underside of the caudal knob into the substrate material while walking along, leaving a continuous sinusoidal trace for considerable distances. All *Nephrurus* species observed in life, and both *Underwoodisaurus* species may wave the tail slowly in a raised position when threatened. The action in *Nephrurus* species with small tails is much less distinct.

When restrained in the hand, *Nephrurus* species tend to remain still for a minute or so before trying to make a dash for escape. Prior to this they almost invariable start waving the caudal knob and tail tip with an increasing amplitude until the dash is attempted.

The defensive display behaviour of repeated slow push-ups, as seen in *Nephrurus* species (Bustard, 1967b) has not been observed in *Underwoodisaurus*. Sand basking has been observed in both *Nephrurus* and *Underwoodisaurus*. Sand basking in captivity involves the gecko laying flat on the warmed substrate with limbs spread out and snout also on the substrate. A particular form of sand basking behaviour has only been seen in *N. asper* where, after emerging from its burrow, the gecko scrapes away the substrate surface (which would cool rapidly after sunset) and lays flat on the surface with limbs outstretched. As that area cools it will dig out a little more substrate at one side and move across to the newly exposed substrate, slowly moving sideways in an arc, and sometimes completing a full circle if undisturbed. The depth of substrate removed is approximately equivalent to the body height of the gecko.

When alarmed, both sexes of *Nephrurus* species and *U. milii* vibrate the tail tip on or near the substrate. During an aggressive territorial display the adult male *U. milii* gecko's tail vibration may be of sufficient force to throw sand grains many centimetres.

Although *U. milii* is abundant in certain areas (Ehmann, 1992), only one adult male is ever found in any aggregation in the wild, unless the site is particularly large. Also outside of the breeding season, males may sometimes be found closer together (personal observation). Male *U. milii* have been found in aggregations in the wild (Kearney, et al., 2001), but in captivity they are highly intolerant of each other, particularly in the breeding season.

However, male *U. milii* were found to cohabit peacefully with male geckos of many other species, including *Oedura tryoni, Oedura robusta, Heteronotia binoei, Gehyra variegata,* and *Nephrurus* but not with
U. sphyrurus. Males of all other *Nephrurus* species observed (*N. amyae, N. asper, N. deleani, N. laevissimus, N. levis, N. stellatus*) will cohabit peacefully with conspecific males. When a male and conspecific female (of *N. amyae, N. asper, N. deleani, N. levis, N. stellatus, U. milii* and *U. sphyrurus*) were housed together they would usually use different refugia provided suitable alternative sites (such as rock crevices or cavities under logs or bark) were made available. Behavioural observations related to reproduction are discussed in Chapters 4, 5.

3.4 Discussion

3.4.1 Sex Ratios

Any collection bias towards larger specimens is not thought to be relevant here because female specimens did not significantly outnumber male specimens in any *Nephrurus* or *Underwoodisaurus* species (Sections 3.3.2, 3.4.1). As the sex ratio for all except one *Nephrurus* and *Underwoodisaurus* species is not significantly different from 1:1, the skewed sex ratio for *N. vertebralis* (2.43:1, males: females) is most likely spurious. Factors such as collector bias, time of day or season when collection is made and differential mortality can cause considerable apparent variation to sex ratios (Beutler, 1981; Delean, 1982; Schoener, 1983; Greer, 1989; Page, 1995). Sex ratios varying from 1:1 to 3:1 have been recorded in the field for *N. stellatus* collected on different occasions (Galliford, 1981). There is no evidence in the reproductive biology, such as population structure or temperature-dependent sex determination, of *Nephrurus* and *Underwoodisaurus* geckos to suggest that the hatching sex ratio should be different from the usual 1:1 generally found in lizards (Blair, 1960; Turner, 1977; Schoener, 1983; Greer, 1989).

Territorial males tend to be larger than females, but intense competition for mates increases male mortality in some lizards (Trivers, 1976). Among territorial lizards there is generally a negative correlation between sex ratio (male/female) and SSD (Stamps, 1983), implying that relatively larger males are more aggressive. In this study the sex ratios (male/female)

for *Nephrurus* and *Underwoodisaurus* species, including live and preserved specimens, are not significantly different at 1.11:1
(N = 705) and 1.13:1 (N = 286) respectively (Chapter 4). One reason why there is no SSD in *Underwoodisaurus milii* may be because the male-male competition is minimal, or alternatively, because there is a selective advantage for increased size in females approximately equivalent to the selective advantage for increased size in males. This latter possibility is supported by the fact that intense competition occurs between males (personal observation). However, this intense competition is not sufficiently great to alter the sex ratio.

3.4.2 Snout-Vent Length and Sexual Size Dimorphism

Adult females are significantly larger than adult males in twenty-six out of 43 gecko species, in 13 species the males were larger, and in four species the maximum size was identical for both sexes (Fitch, 1981). The range of the female to male SVL size ratios is 84-128% (Fitch, 1981). Sexual size dimorphisms of 128% in *Sphaerodactylus goniorhynchus* and 139% in *Aristelliger georgeensis* have been reported (both with larger males) (Schwartz and Henderson, 1991). No diplodactyline species were included in the Fitch analysis. A SSD of 120% (female to male ratio) in Western Australian *Nephrurus* species was 'the largest recorded in geckos' (How et al., 1990).

Much speculation surrounds the occurrence of SSD in certain reptile species and the reasons why there is so much variation, even within closely related species (Fitch, 1981; Shine, 1985b). One variable which has frequently been linked to SSD is that of body size with larger species tending to have larger males than females and smaller species tending to have larger females than males (Rensch's rule) (Colwell, 2000). However, this rule is not followed in *Nephrurus* and *Underwoodisaurus* species, which, although intermediate to large in size compared to most geckos, do not have any male size-biased species. If the competition-avoidance hypothesis were the sole or major factor involved in gecko SSDs, then it might be expected a priori that there would be approximately equal numbers of species with larger males as with larger females, which is not the case for the Gekkonidae (Fitch, 1981) nor for the *Nephrurus* /*Underwoodisaurus* group (Table 3.2). The argument for the competition-avoidance hypothesis is also weak because the majority of SSDs in

geckos are less than 7% (Fitch, 1981) and therefore any difference in prey size is slight, and non-existent during the growing period (in this study juvenile males and females of *N. levis* and *U. milii* grow at similar rates under similar conditions). The SSD range for *Nephrurus* geckos found in this study is 110-133% (mean 120%) (Table 3.2), which, in conjunction with the large size of these species suggests strong selective pressure towards large females.

The intrasexual selection hypothesis suggests that SSD is more directly related to reproductive strategy. In species with male-male combat, as in highly territorial species, larger males tend to win intraspecific contests and thus larger males are selected (Fitch, 1981; Shine, 1985b). However, this hypothesis does not explain why some territorial males are not larger than the females nor the fact that in the majority of gecko species it is the female that is larger. The larger offspring hypothesis suggests that larger offspring at birth/hatching are more likely to survive to maturity (Shine, 1978a, 1985b). Therefore, if a female can produce larger offspring, she can increase her reproductive efficiency and selective pressure would favour larger size in females. The fact that all the *Nephrurus* species have significantly larger females than males is consistent with the third hypothesis that increased female size correlates with increased egg size and offspring size (Chapter 5). *Nephrurus amyae* is biogeographically the most arid adapted species of the genus, and is also the largest *Nephrurus* species (Table 3.2, Appendix 1, Table A1.1). It has spinose dorsal tubercles that probably assist in providing an effective moisture barrier (Lillywhite and Maderson, 1982; Figure 1.1). Although the primitive *Nephrurus* group (*N. amyae, N. asper, N. sheai, N. wheeleri*, the four species with the plesiomorphic phalangeal formula, Greer, 1989) has the largest geckos, there is no significant difference in the mean values for SSD between the primitive *Nephrurus* group (119.7%) and the derived *Nephrurus* group (*N. deleani, N. laevissimus, N. levis, N. stellatus, N. vertebralis*, with a further-reduced phalangeal formula) (120.0%), or between the *Nephrurus* group (119.8%) and the *Underwoodisaurus* group (115.8%).

There is no complete explanation for the sexual body size dimorphism, but its consistency among all *Nephrurus* species suggests either a possible phylogenetic constraint or an environmental factor applied to all species. The phylogenetic constraint theory is not supported by the weight of evidence. Firstly there is considerable intrageneric variation in SSDs (Fitch, 1981) and secondly *U. milii* (Table 3.3) and five of the six available

diplodactyline out-group species show no SSD (Appendix 1, Table A1.12). One hypothesis proposed here is that arid species produce larger eggs than mesic species (Chapters 4, 5). Females that lay porous, flexible-shelled eggs may have an advantage in arid environments if they lay eggs with a larger volume to surface area (Chapter 5). Because such a selective pressure acts only on females, and because it could be critical for survival in arid environments, it is likely to favour the development of significantly larger females than males (33% larger in *N. deleani*, Table 3.2).

The anomalous position of *U. milii*, with no significant sexual body size dimorphism ($T_{(2)98}$ = -1.15, P = 0.125) and territorial males may be explained on the basis of a combination of selective pressures. Larger males are selected because of their territorial behaviour (Shine, 1994) and larger females are selected because they can produce larger eggs. Also the mesic *U. milii* may experience less intense selection pressure to produce larger eggs than arid *Nephrurus* species (Chapter 5). Explanations for possible selective advantages of territorial behaviour include defence of food, refugia, basking sites, mates and nesting sites (Stamps, 1983). In captivity, males of *U. milii* are strongly territorial, thus selecting for an increase in size of the males (Zug et al., 2001). A test of the hypothesis is available in the comparison of *U. milii* with the non-territorial congener *U. sphyrurus*, in which females are significantly larger than males (Table 3.2). The fact that *Nephrurus* males are significantly smaller than females suggests that there is no differential predatory pressure against smaller size, otherwise sex ratios might be different and both males and females would tend to be large. Small males in *Nephrurus* species suggests that males are not territorial and do not engage in male-male combat (as confirmed by captive observations). The males of *Nephrurus* species may be non-territorial because most live in arid regions and at low population densities, so that male-to-male combat is of relatively less significance. In contrast, *U. milii* (unlike *U. sphyrurus*) is often locally abundant, (Ehmann, 1992; personal observation). I have been unable to find reference to any male territoriality among carphodactyline geckos; if this is correct, territoriality in *U. milii* may be considered a secondary acquisition. The fact that the habitats (Appendix 5) and life histories of both *U. milii* and *U. sphyrurus* are similar (nocturnal, terrestrial, similar sized, opportunistic arthropod feeders) suggests that there is not likely to be a major differential in the size selective pressures on the females.

3.4.3 Tail Measurements and Functions

There is positive allometry of tail growth (as % SVL) in adult males of *U. milii* ($y = 0.252x + 57.7$) compared to slightly negative allometry in adult females ($y = -0.136x + 81.0$). The mean relative tail length is significantly greater among males than females in five species, representing primitive and derived *Nephrurus* and *Underwoodisaurus* groups (*N. asper*, *N. deleani*, *N. levis*, *N. wheeleri* and *U. milii*) (Table 3.2, Figures 3.4, 3.5, Appendix 1). This small but consistent dimorphism may be related to the development of hemipenes (R. Shine, personal communication). As the hemipenes grow they increase the gap between the cloacal aperture and the basal tail crease or autotomy plane. Thus, an increased male tail length may result from the pressure of development of the hemipenial apparatus acting on the immediate post- pygal joints and vertebrae. Some support for this theory is provided by the fact that juveniles do not show this SD and the rate of tail growth in juveniles is almost isometric. However, the fact that mean relative male tail growth is greatest in species with longer tails (e.g. 3.6% of SVL for *Nephrurus* species and 6.4% of SVL in *U. milii*) suggests that the growth extends over the length of the tail and is not a quantum increase that might be expected from hemipenial development. Another possibility is that increased tail length is related to the longer hind limbs found in the males of some species (section 3.4.7).

A significant character within the primitive *Nephrurus* group is the lack of a caudal autotomy plane in *N. asper*, a unique occurrence among the Gekkonidae (Holder, 1960; Bauer, 1986, Greer, 1989). The fact that all specimens in the *N. asper* group examined in this study (N =158) had original tails (except two with surgically removed tails), suggests that both *N. amyae* and *N. sheai* are also without a caudal autotomy plane. Radiography of both species confirmed the stability of this character and the close affinity of the *asper* group species by showing the absence of a caudal autotomy plane. In contrast, *N. wheeleri* (also in the primitive *Nephrurus* group) follows the rest of *Nephrurus* in possessing a basal autotomy plane. The relative tail length SD (Table 3.2, Figures 3.4, 3.5,) is present in both longer and short-tailed species (Table 3.2). In *N. deleani* there is a basal autotomy plane, as evidenced by a crease in the skin and by one specimen that had lost its tail after capture (N = 15, no specimens with regrown tails were seen). The presence of a caudal autotomy plane was confirmed by radiography of one male, one female and one juvenile specimen.

The mean relative tail dimensions (sum of length, width and height), varied by a factor of 55% above the minimum in males compared to 63% in females. The large variation in males probably reflects nutritional status; the larger variation in females may be related to the intermittent energy requirements of egg production.

Tail autotomy among *Nephrurus* is very low at 0.6-8.6% (Pianka and Pianka, 1976; Delean, 1982; Greer, 1989). The low autotomy rate may be interpreted as low predation rate or robustness of the autotomy apparatus, or both. Autotomy rates may be reduced in species with actively functional tails (Greer, 1989) but in many gekkonid species this is not the case (Bauer and Russell, 1994 (1995)). The tail autotomy rates for *Nephrurus* in this study (Table 3.6) are comparable to the published rates. Lack of territoriality among *Nephrurus* species suggests that the tail losses are related mainly to interaction with predators rather than conspecifics. There is no correlation of autotomy rates and tail size; with relatively high and low autotomy rates being found among large-tailed species as well as small-tailed species.

Territorial behaviour of *U. milii* may account for a higher autotomy rate in this species but it is evidently only part of the answer. Males have only a marginally higher autotomy rate (16.4%, N = 67) than females (15.9%, N = 63); also the non-territorial *U. sphyrurus* has a higher rate at 19.2% (N = 26). Another possible explanation for the increased rate of tail loss in *Underwoodisaurus* is the highly developed lure behaviour. The tails in *U. milii* are long and broad (Figures 1.10, 3.1) indicating an important lipid storage function (Chapter 6), which could make the tails more attractive to potential predators. Males do not lose their tails easily (personal observation), but this does not stop the relatively high tail loss rate compared to *Nephrurus*.

The presence of a very small, non-autotomous tail in the primitive *Nephrurus* group is a derived state that matches the tropical distribution with reduced lipid storage requirements. Both *Underwoodisaurus* species are large-tailed and have a southerly distribution, suggesting that storing more lipids in the tail enhances survival during the longer and colder southern winters. However, the distributions of small-tailed *Nephrurus* species in the north (*asper* group) and in the south (*N. deleani* and *N. stellatus*) and large-tailed species (*N. levis* and *N. wheeleri*) in the north and south suggest a bimodal solution to the problem

of energy storage. All *Nephrurus* and *Underwoodisaurus* geckos, male and female, store significant lipids in the abdominal fat body when conditions are favourable, suggesting that the abdominal fat body is the primary defence against adverse conditions, and that it may be critical to the survival of geckos that have lost their tails. Both lipid reserves are likely to contribute towards reproductive energy requirements, especially in the female (Chapters 5, 6).

The mixed results for SD of the caudal knob (significant in four out of nine species), caudal knob scales and isthmus size show little pattern in their relationship relative to polarity and phylogeny and may be stochastic variations rather than significant results. Larger caudal knobs in some adult males compared to females suggest a possible connection between the caudal knob and reproductive function, however, this is countered by the fact that the relative size of the caudal knob decreases with increasing snout-vent length throughout life.

3.4.4 Head Size

Males have relatively wider heads than females in seven species; *N. asper, N. laevissimus, N. levis, N. sheai, N. wheeleri, U. milii* and *U. sphyrurus* (Table 3.3, Figure 3.6). Relative head length is also significantly larger in males compared to females in five species, *N. amyae, N. laevissimus, N. levis, N. sheai* and *U. milii* (Table 3.3) and head depth is also greater in males than females in five species, *N. laevissimus, N. levis, N. sheai, U. milii* and *U. sphyrurus*. Thus, examples of increased male head dimensions were found in the primitive *Nephrurus*, the derived *Nephrurus*, and *Underwoodisaurus* groups.

There are two commonly accepted reasons for larger heads in reptiles. One suggestion is that larger prey items can be overpowered and swallowed (Savitsky, 1983). The other relates to male - male combat, where territorial males that oust other males from their territory often have larger heads (and/or larger bodies) than the females (Fitch, 1981; Carothers, 1984; Shine, 1990; Shine, 1994; Stamps et al., 1994). As territoriality is unknown in *Nephrurus* and there is no reason to believe that there is feeding partition between males and females, the reason for any SSD of the head is not obvious. An alternative explanation is that male lizards with larger heads have a selective advantage because they have to grasp the female with their jaws during copulation (Chapter 4). This argument is weak because such behaviour is almost universal among species with and

without the sexual head size dimorphism. Another alternative is that males with larger heads have a selective advantage because they are better able to protect the female from smaller males. However, there is no evidence that mate protection behaviour occurs in either *Nephrurus* or *Underwoodisaurus*. The finding of larger heads in males of the majority of species suggests that territoriality may be a recently lost plesiomorphic character.

Although there is no significant sexual body size dimorphism in *U. milii*, there is a strong relative head width dimorphism (Table 3.3, Figure 3.6). This combination suggests that there is, or has been, evolutionary pressure in the direction of increasing relative body size in males as well as increased relative head size in males. Another possibility is that the females of *U. milii* have not experienced the selective pressures to increase body size and head size as in *Nephrurus* species. Intraspecifically there is a close correlation between head width and snout-vent length, with a negative allometric growth rate for head width relative to SVL for juvenile males, juvenile females and adult females but a positive allometric growth rate for adult males (Figure 3.6, Appendix 1, Table A1.10). This difference in head growth rate suggests that the presence of larger heads in males is associated with reproductive maturation.

3.4.5 Transiliac Width

The mean relative transiliac measurement is greater among adult females than males in *N. amyae*, *N. laevissimus*, *N. vertebralis* and *U. milii* (*i.e.*, representing all three phylogenetic study groups) (Table 3.4, Figure 3.7). Dystocia, or egg retention, has been recorded in lizards, including geckos (Harvey, 1983; Girard, 1993; Wagner and Lazik, 1998). If there is selective pressure to increase the size of eggs, then there may also be a concomitant selective pressure to increase the dimensions of the pelvic outlet in females. If eggs close to the limits of pelvic dimensions are oviposited, dystocia may be expected to occur occasionally (Chapter 5).

3.4.6 Cloacal Tubercles

Cloacal tubercles are present in rudimentary form in juveniles and adult females, but undergo further development at sexual maturity in males of all *Nephrurus* and *Underwoodisaurus* species. The relative transtubercular measurements are significantly greater in the adult males of all species studied (except *N. amyae* and *U. sphyrurus*) compared to juveniles and females (Table 3.4, Figure 3.8). Some variation in morphology of the cloacal tubercles in carphodactyline geckos has been described (Bauer, 1986), but there is no consensus on their function. The pointed shape, position (adjacent to the cloacal opening) and near vertical orientation of the postcloacal tubercles suggests they function in gripping the female and maintaining position during copulation. Another suggestion is that the tubercles 'hold open the female cloaca during copulation' (Henkel and Schmidt, 1995). Adult male pygopodids have a single, smoothly curved, hook-like tubercle situated adjacent to the cloaca (personal observation) which may be able to hold open the female cloaca, but *Nephrurus* and *Underwoodisaurus* species have a tuft of tubercles situated several mm away from the male cloaca (Figures 3.2, 3.3), indicating that the grip location would also be several mm away from the female cloaca and possibly in the region of the ischial bones.

The transtubercular width dimorphism occurs despite the fact that adult females develop significantly wider pelves (relative transiliac width) compared to males in four species (Table 3.4, see below). The increased development in males of cloacal tubercles at sexual maturity suggests a possible connection with reproductive function (Chapter 4).

3.4.7 Escutcheon and Preano-Inguinofemoral Tubercles

The function of the escutcheon is not certain, but the location, topography, waxy appearance and lack of holocrine type pores in males, suggest a possible pheromonal role, perhaps assisting sexually receptive females to locate males (Cooper and Steele, 1997). Another possibility is that any secretion could be used as a territorial marker (Henkel and Schmidt, 1995), possibly to inhibit the entry of conspecific males into a burrow or defended territory.

The presence of preanal and/or femoral pores is widespread among lepidosaurians (agamids, Ehmann, 1992; amphisbaenians, Dixon and Soini, 1975; iguanids, Alberts, 1989; pygopodids, Cogger, 2000; lacertids, Obst et al. 1988) suggesting this is a primitive state (Kratochvíl and Frynta, 2002). Males of the eublepharid gecko *Eublepharis macularius,* which have femoral pores (Minton, 1966), are capable of distinguishing males from females by the detection of pheromones (Mason, 1992), thus supporting the possibility of *Nephrurus/ Underwoodisaurus* species producing pheromones. The escutcheon in *Nephrurus* species (unlike *Underwoodisaurus* species) does not change with season, appears to be inactive and possibly involves only a change in size of scales during embryonic development, possibly due to proximity to the umbilical scar region (which frequently has enlarged scales in many gecko species, personal observation). The post-umbilical position of the escutcheon in *Nephrurus* and *Underwoodisaurus* species suggests that it may not be homologous with the highly developed preanal glandular scales found in eublepharid geckos (Taylor and Leonard, 1956) *e.g.*, *Coleonyx* (Maderson, 1968) and in sphaerodactyline geckos *e.g.*, *Gonatodes* (Maderson, 1967). In comparison, the Australian endemic *Carphodactylus laevis*, which is regarded as the most primitive of all carphodactyline species (Kluge, 1967a), is characterised by the presence of weakly developed preanal organs (Bauer, 1990b). If the structures were homologous, the presumed primitive status of *Underwoodisaurus* species would be supported. The presence of a preanal patch of pores is primitive in the Carphodactylini (Kluge, 1967a, 1983). A preanal patch of tubercles, assumed to be a 'remnant of pores', is generally present in *Nephrurus* (Kluge, 1967a). However, I found no 'patch' of tubercles or preanal pores, or anything like the escutcheon found in some male diplodactyline, sphaerodactyline and eublepharine geckos, in either *Nephrurus* or *Underwoodisaurus* geckos. The specialised escutcheon region found in *U. milii* (Figure 3.1) is more highly developed than in *U. sphyrurus*, in which it is also better developed than in any of the *Nephrurus* species (Chapter 4). These facts suggest that the escutcheon function is a primitive characteristic that has been lost in *Nephrurus* species and is less important in *U. sphyrurus* than *U. milii*. The function of the escutcheon is suggested by the fact that only *U. milii* has a moderately well developed escutcheon and male combat related to territoriality. The intolerance of male *U. milii* to both *U. milii* and *U. sphyrurus* males, and tolerance for other male gecko species, can be explained on the basis that the escutcheon produces an identification pheromone. The

escutcheon must be active in both *Underwoodisaurus* species and the pheromone must only be produced by males and must be different from that in the tolerated species, but very similar in both *Underwoodisaurus* species. It also explains why intolerance is reduced in cold weather when pheromonal activity would be reduced.

Whether either of the two specialised types of ventral scales found in *Nephrurus* and *Underwoodisaurus* geckos is homologous with the preanofemoral pores of other gekkonid and non-gekkonid lepidosaurians is debatable. The differing morphology, location and arrangement of the preano-inguinofemoral tubercles found in certain *Nephrurus* species (Figure 3.2) suggests they may not be homologous with the post-umbilical patch found in male *Underwoodisaurus* geckos (Figure 3.1). For the same reasons, the preanal pores of other geckos (*e.g.*, certain *Saltuarius* species) may not be homologous with the umbilical patch of specialised scales found in *Underwoodisaurus* geckos. It is probable that *Nephrurus* has lost the primitive character of preanal pores (Chapter 2). One possible explanation for such a loss is found in the natural history of these species. All *Nephrurus* have distributions covering arid or semi-arid areas (Figures 2.1 - 2.9) where the dry or sandy substrate frequently moves. If the function of the pores is to delineate territory, then preanal pores would be of little or no use. Another possibility is that *Nephrurus* species have lost their pores because they provided location evidence to chemosensory predators such as snakes. *Underwoodisaurus* species, on the other hand, which have retained the aggressive male territorial behaviour and generally live in less arid environments, might find a territorial marking system of survival value.

3.4.8 Limb Length

All four species with significant forelimb SSD (*N. amyae*, *N. sheai*, *N. vertebralis* and *U. milii*), are also found among those species with significant hind-limb dimorphism (*N. amyae*, *N. asper*, *N. sheai*, *N. stellatus*, *N. vertebralis*, *N. wheeleri* and *U. milii*) representing all three study groups (Table 3.5, Appendix 1, Tables A1.1-A1.11). Increased forelimb length is presumed to be linked to increased hind-limb length in maintaining optimum balance and movement. The longer hind-limbs are probably important in pursuit and capture of prey, escape and threat behaviours and leaping *e.g.*, from one rock to another. Long hind-limbs are also important for the slow push-ups as part of a threat

display and for sand-basking behaviour in *Nephrurus* species. The question of why the hind-limbs are relatively longer in males is debatable, as all the activities mentioned above are similar in males and females. The only sexually related activities involving the legs that have been observed relate to male-to-male combat, where the legs are important for helping maintain stability (in *U. milii* only), and in courtship and mating rituals. Male geckos tend to become sexually mature at a smaller size than females (Chapter 4) and a very small adult male gecko sometimes appears to struggle to maintain its copulating position with a large female (Wilson and Knowles, 1991). Another possibility is that males may range more widely, increasing exposure to predators; consequently longer legs may help in eluding capture.

The selective advantage of longer legs in *Nephrurus* species may be less than in *Underwoodisaurus*. In *Nephrurus* species, where male-to-male combat is unknown, males would not have relatively longer hind-limbs compared to females if combat were the only factor involved. The fact that adult male *U. milii*, which are close to females in size, have longer hind-legs but also engage in male-to-male combat, may suggest multifactorial selection pressures. Another explanation is that longer hind-limbs and combat were linked as primitive characters in the past but that now only the longer hind-limbs survive. This view is supported by the finding that all the primitive *Nephrurus* species are included in the longer hind-limb group. Males of
N. stellatus and *N. vertebralis* are also included in this group. Females are more robust in terms of body width and therefore the shoulder and hip joints may be more deeply embedded below the skin (from where limb measurements were made). However, the fact that the legs are not longer in males of two species (*N. laevissimus* and *N. levis*) where large numbers were measured, suggests that possession of longer legs in the males of some species is not an artifact.

If males have significantly longer tails than females it would also be expected (other measurements unaltered) that this would shift the animals centre of mass towards the hind-limbs. However, no significant correlation was found among species for the limb length sex dimorphism and the tail length sex dimorphism. There was also no significant correlation of snout-vent length and limb length. All these factors taken together (including the

difference in forelimb and hind-limb dimorphism) suggest that there is no systematic scaling factor (Jaksic, 1981) involved in the limb length dimorphism.

3.4.9 Subdigital Scales

Whether the increased subdigital lamellae numbers are related to the longer legs or to some sexual or other behavioural characteristic is unknown. Many gecko descriptions include counts of subdigital lamellae but I have only found two moderately extensive studies of lamellae numbers in geckos (Hecht, 1952; Bustard, 1965b) without mention of SDs. A study of subdigital lamellae in anoles (Collette, 1961) showed a SD in lamellae numbers with more lamellae in males than females. However, the evidence suggests that a major reason why male anoles have more lamellae is because they grow larger and therefore require greater adhesive capacity. Selection for increased lamellae numbers in larger geckos has also been found in *Aristelliger* spp. (Hecht, 1952). Lamellae numbers were also increased in arboreal compared to terrestrial anole species (Collette, 1961). In *Underwoodisaurus* geckos both sexes dig burrows and neither sex size dimorphism nor arboreality apply, so any reasons for the dimorphism in lamellae numbers must be sought elsewhere.

Many arboreal and saxicoline geckos have large series of transversely arranged scales forming very narrow lamellae, presumably to increase the surface contact area and take advantage of more surface energy to increase adhesion to the substrate (Russell, 1986; Bauer and Good, 1986; Autumn et al., 2000, 2002). It is possible that male *Underwoodisaurus* have increased their grip potential by increasing the numbers of subdigital lamellae, although terrestrial geckos generally do not have any specialised surface-energy utilising fibrillar ultrastructure commonly found in arboreal species (Russell, 1986). I have observed that both *U. milii* and *N. levis* are adept at climbing vertical surfaces such as brick walls and rock surfaces, but they use their sharp recurved claws for climbing and cannot cling to smooth surfaces. There is some evidence that *Nephrurus* and *Underwoodisaurus* geckos are secondarily padless. Many related out-group species such as most *Diplodactylus* and *Oedura* species have expanded and divided apical subdigital scansorial lamellae suggesting this may be the primitive state. The fact that *Underwoodisaurus* species have reduced (non-scansorial) but still divided apical subdigital

lamellae supports this conclusion. If this is the case then any SD of lamellae numbers may be only a reflection of past rather than present selection pressures.

Chapter 4

Reproduction in *Nephrurus* and *Underwoodisaurus*

4.1 Introduction

4.1.1 Reproductive Biology of *Nephrurus* and *Underwoodisaurus*

Reptiles exhibit a wide range of reproductive modes that impact reproductive success, *e.g.*, some species may reproduce many times in one year while others reproduce annually or less frequently. Also some species are viviparous while others are oviparous (Porter, 1972; Bellairs and Cox, 1976; Goin, et al., 1978). As a general rule, fecundity is determined by a combination of phylogenetic constraints and natural selection pressures (Smith and Fretwell, 1974; Stearns, 1976). Although lizard species demonstrate a wide range of reproductive modes affecting fecundity (Stamps, 1983; Greer, 1989), a relatively narrow range of reproductive strategies is found among the Gekkonidae. Oviparity is the norm and presumed primitive state for all gekkonoids, although viviparity occurs in two carphodactyline lineages (Bartmann and Minuth, 1979; Miller, 1984; Henkel and Schmidt, 1995; Gill and Whitaker, 1996).

Eleven modes of reproduction are recognised in Australian reptiles (Heatwole and Taylor, 1987), only four of which occur in Australian geckos (King and Horner, 1993). For example parthenogenesis may occur *e.g.* in some *Heteronotia* populations (Whittier et al. 1993), also, mating may occur in spring or early summer (most species), or in the autumn *e.g.* in *Christinus marmoratus* (King, 1977), clutches may be of either one egg *e.g. Gehyra variegata* (Dell and Chapman, 1978) or two eggs (most species, Greer, 1989) and there may be from one to several clutches per year (King and Horner, 1993). Sperm retention by the female also occurs in some species *e.g. Nephrurus asper* (Couper 1996). Hatching is usually early to late summer (Heatwole and Taylor, 1987).

4.1.2 Sexual Maturation and Reproduction

Little is known about the reproductive biology of *Nephrurus* and *Underwoodisaurus* species in the wild, but their reproductive cycles can be inferred from measurements of gonads taken at different times of the year (*e.g.*, Cunningham, 1993; Mendez-de la Cruz, et al., 1993) and by examination of histological sections of gonads (*e.g.*, Boyd, 1940; Miller, 1948; Chiu and Maderson, 1975). Correlation of gonadal status with date of collection is a standard method of determining the reproductive cycles of reptiles from museum specimens (*e.g.*, MacAvoy, 1976; Shine, 1985b; Simbotwe, 1985; Guraya, 1989; How, et al., 1990). One problem with museum material is that most specimens are collected opportunistically over many years, *e.g.*, from hot-dry as well as cool-wet seasons and more often during the summer than winter. Thus not all months are represented in museum collections for most Australian geckos, including *Nephrurus* and *Underwoodisaurus*. Additionally, a natural asynchrony of reproductive cycles may be expected when collections are made from a variety of populations at different latitudes, altitudes and habitats. In this chapter, I analyse reproduction in *Nephrurus* and *Underwoodisaurus* geckos based on dissection of museum specimens, but with the above limitations in mind.

Sexual maturity is generally defined as the first point in an organism's life cycle when mature gametes are produced. When dealing with preserved material, sexual maturity is more conveniently defined as the SVL when the size of the gonads increases significantly (Campbell and Reece, 2002; King and Horner, 1993). As the ovaries develop, the follicles become more spherical and there is increased deposition of cream to yellow coloured yolk (Boyd, 1940; MacAvoy, 1976). These changes correspond to large increases in follicular DNA, RNA and protein (Sharma and Grewal, 1996). There is also a significant increase in size of oviducts (Palmer and Guillette, 1991) and presumably development of any secondary sexual characters at the onset of sexual maturity.

In male reptiles there is an increase in size of testes relative to body size at sexual maturity by a factor of up to ten in some species (King and Horner, 1993; How et al., 1990). One problem in defining minimum size at sexual maturity is that this size may vary from one individual to another, from one season to another and from one population to another. Therefore, the larger the sample size the smaller the minimum size at sexual maturity is

likely to be compared to maximum snout-vent length. The epididymides and vasa deferentia also increase in size significantly and there is further development of any sexual dimorphisms at the onset of sexual maturity.

The most comprehensive study of reproduction in *Nephrurus* and *Underwoodisaurus* species, based largely on museum specimens of three species, with information on another four species (How et al., 1990) (Figure 4.1) found that:

1. there is a significant sexual size dimorphism in all species, with females being 'around 20 per cent' larger than males.

2. male *Nephrurus* species reached sexual maturity at between 82% and 89% of the size of females at maturity and at 92% for *U. milii*.

3. the sex ratio was near parity for adults.

4. vitellogenesis and spermatogenesis in *Nephrurus* species probably occur in any month if conditions are favourable.

5. the testes of *U. milii* increase in size over-winter and vitellogenesis, ovulation and oviposition occur mainly in spring and summer.

6. sequential clutches occur in all species.

Nephrurus and *Underwoodisaurus* species have many morphological, reproductive and behavioural differences even though they are similar in general body size, shape and diet. Possible explanations for the different radiations of the two genera lie in the differences in the eggs (Chapter 5) and in reproductive strategies (see below).

The larger female body width and greater SVL compared to males are both characters that allow a larger clutch mass. The wider pelvic bones in females (Chapter 3) facilitate the passage of larger eggs. The increased size of postcloacal tubercles in males compared to females are probably related to the need to maintain position and grip during copulation. It is also possible that the tubercles assist in holding the female cloaca open (Sections 2.4, 3.3.3, 4.4.1).

With a fixed clutch size of two (Shine and Greer, 1991, Chapter 5), female geckos can adjust the rate of reproduction only by varying the number of clutches per season (Shine and Greer, 1991; Doughty, 1997). However, some geckos are very long lived, even in the wild (Anastasiadis and Whitaker, 1987; Barwick, 1982; Thompson et al., 1992) and can thus increase reproductive output over a lifetime. Individual female geckos can also increase their reproductive success by increasing their investment in each of the progeny by producing larger or better-adapted young (Smith and Fretwell, 1947).

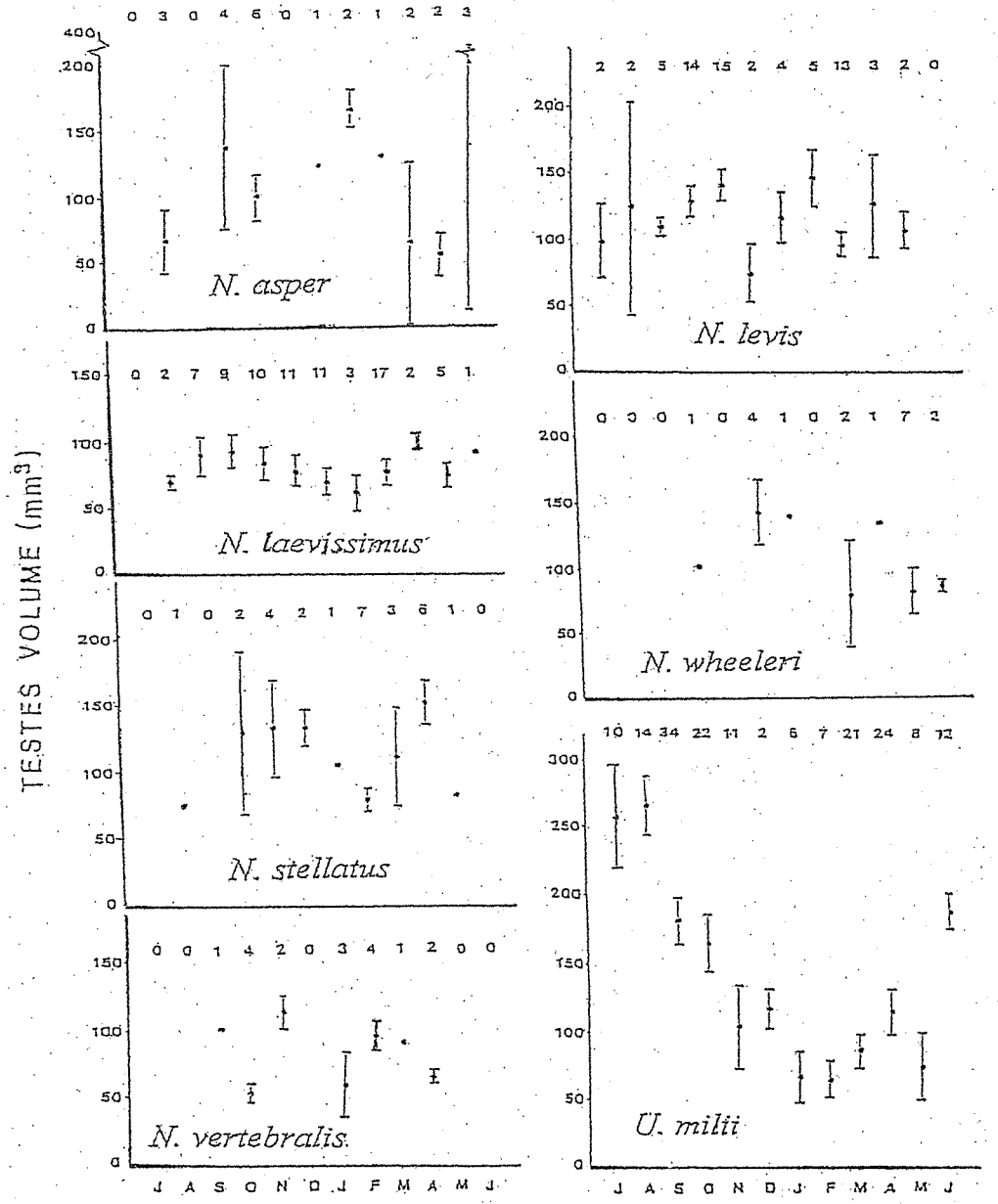

Figure 4.1 Reproductive cycles of male *Nephrurus* and *Underwoodisaurus* species as shown by testis size through the year. The numbers above each month are the numbers of geckos sampled (based on How et al., 1990). *'N. asper'* represents mainly a combination of *N. amyae* and *N. sheai*.

Sperm storage occurs in *U. milii* (How et al., 1990) and *N. asper* (Couper, 1996). Also a gravid female *N. deleani* collected in late April remained gravid through the winter (Harvey, 1983). Confirmation of sperm storage or egg retention by female *Nephrurus* and *Underwoodisaurus* geckos would provide evidence of additional mechanisms for increasing fecundity (by increased number or viability of clutches per season, and allowing breeding in unusually short seasons or when there is asynchrony of male and female reproductive cycles (Chapter 4).

Anoles may mate more than once daily during the summer and alternate the use of the left and right hemipenes (Tokarz, 1988). Very little is known about mating in Australian geckos (Greer, 1989), with no detailed descriptions available for *Nephrurus* or *Underwoodisaurus* species. No information is available on frequency of mating. Mating occurs at temperatures down to 19 °C in *N. stellatus* (Page, 1995), 21.8 °C in *N. levis* (Annable, 1992) and 24 °C in *N. asper* (Annable, 1992). Mating occurs in the wild in late August for *N. stellatus* (Page, 1995). Temperature, photoperiod and nutritional status are probably the major factors that control reproduction in *Nephrurus* and *Underwoodisaurus* spp., as they do in other reptiles (Licht, 1971).

Nephrurus and *Underwoodisaurus* species may multiple-clutch (Annable, 1992), but frequency of reproduction may be influenced by age and nutrition of female, nesting opportunities, social factors, weather and suitability of substrate (Stamps, 1983).

I aimed to examine reproductive characteristics of *Nephrurus* spp. and *Underwoodisaurus* spp. to identify fundamental characteristics that may facilitate the reproductive success of these species and enable detailed comparison with other gecko species. The questions of particular importance to understanding the reproductive strategies of *Nephrurus* and *Underwoodisaurus* geckos are:

1. How do species of *Nephrurus* and *Underwoodisaurus* maximise their reproductive success while producing only two eggs per clutch?

2. How are gonadal cycles of males and females correlated?

3. Are the reproductive cycles compatible with over-winter sperm storage?

4. What differences in reproductive strategy are there between tropical and temperate species?

5. What are the differences in reproductive strategy between desert and mesic gecko species?

I used a multifaceted approach to address these questions by utilizing captive and preserved museum specimens.

4.2 Methods

4.2.1 Acquisition of Live Specimens and Husbandry

Adult specimens of *N. amyae* (N = 4), *N. asper* (N = 4), *N. levis* (N = 18), *N. stellatus* (N = 20), *N, wheeleri* (N = 1), *U. milii* (N = 32) and *U. sphyrurus* (N = 6) were collected from the wild and housed under standard husbandry conditions in large vivaria (Chapter 1, Appendix 3). Some attempt was made to provide natural habitat conditions (Appendix 5) to encourage breeding. Some hatchlings were raised to maturity and reproduction repeated as far as the fourth generation for *U. milii*. Locality, date, time, substrate type and temperature were also recorded for wild-caught specimens.

4.2.2 Mating Observations

Observations of captive geckos were made almost daily during the summer, and about twice a week in midwinter. Geckos were housed in large, naturally landscaped vivaria in the laboratory (Appendix 3) and most observations were made in the evening or during the night under subdued artificial light or far red (photographic safelight) illumination. Observations of many behaviours were made using a video camera and still photography for later analysis, but most were recorded in writing with dates, times and temperatures.

4.2.3 Museum Specimens

A pilot study of *Nephrurus* testis measurements showed that the shape and measurements are variable, with the transverse measurements occasionally exceeding the longitudinal measurement. Therefore, I measured the three perpendicular diameters (maximum

longitudinal, maximum cross sectional and minimum cross sectional) *in situ* to determine volumes more precisely. Because ovarian follicles tend to be close to spherical, only one diameter was measured.

A total of 1008 alcoholic specimens of all species in the genera *Nephrurus* and *Underwoodisaurus* were examined and measured (Appendices 1,4). Representative samples of males, females and juveniles collected in spring, summer, autumn and winter (where available) were examined in detail. Because very few specimens have been collected in the winter, there is a paucity of later juveniles of all species and no satisfactory monthly series was available except for *U. milii*. Specimens that had not been previously dissected were opened by a longitudinal abdominal incision and two lateral skin flaps formed to allow measurement of gonads. Macroscopic measurements were taken as described in Chapter 3 and Appendix 1 and measurements from histological specimens were determined using a stage micrometer and calibration photograph at each of the magnifications used. Yolk colour was also noted. Gonads for histological examination were chosen to represent the seasons of the year (where possible) and also some juveniles for comparison with quiescent adult gonads. The whole of the left testis and epididymis (N = 44), or the whole ovary (N = 7) of representative specimens were removed and fixed in buffered formol saline, then preserved in 70% ethyl alcohol.

Fixed gonad tissues were prepared for histological examination using a standard haematoxylin and eosin staining technique (Presnell et al., 1997). Each sample was washed in water, taken through a graded series of ethanol to absolute ethanol, cleared in xylol, wax embedded at 52 °C and sectioned at 5µm. Multiple sections, mounted on standard microscope slides, were stained with aqueous haematoxylin for five minutes. Sections were then washed in water before counterstaining in aqueous eosin. The sections were then washed again in water and dehydrated in alcohol, cleared in xylol and permanently mounted using synthetic resin and a large coverslip before labelling. This technique colour-differentiates nuclei (blue), cytoplasm and connective tissues (pink) and erythrocytes (orange) (Bradbury, 1969; Muir, 1973). Slides were examined and photographed at low, medium and high magnification using a Nikon E800 Photomicrography System. Photos were either processed onto floppy discs (National Photos) or scanned onto computer Zip discs and CDs prior to printing using a Canon BJC 4200 Color Bubble Jet Printer, or prints

were simply photocopied. Magnifications were determined by photographing a stage micrometer scale at each magnification used so that effects of photographic and computer processing could be quantified.

The following measurements and observations were made:

1. Testis volume. The length, breadth and height were measured on both sides to 0.01 mm.

2. Ovarian follicular volume. The diameters of the largest three or four follicles (if detectable) were measured on each side to 0.01 mm. The volume was determined on the assumption that the follicles were spherical.

3. Date of collection and/or death where known.

4. Histology of ovaries. Seven adult ovaries including a juvenile ovary from two species (*N. levis* (N = 4) and *U. milii* (N = 3)) were dissected for histology. The number of ovaries sampled was limited because it was expected to provide limited additional information on the female reproductive cycle.

5. Histology of testes. Forty-four testes (including the epididymis in half of the specimens) from six species as follows: *N. asper* (N = 3), *N. levis* (N = 10), *N. sheai* (N = 5), *N. wheeleri* (N = 7), *N. vertebralis* (N = 8) and *U. milii* (N = 11) were dissected for histology. Samples of large adults were chosen to represent possible seasonal variations, a few juvenile samples were chosen to represent only the immature state. All samples were limited by availability. Sample sizes were inadequate to assess geographic variation.

6. Sperm density was measured by taking sperm counts across the diameters of the epididymis or vas deferens. Sperm density was either very high or very low to absent in nearly all sections, so great precision was not necessary for the determination of reproductive cycles. A circular (*i.e.*, transverse) section of a duct was found, photographed, enlarged and printed. A randomly selected diameter was drawn across the duct and the number of sperm heads touching the line was counted. Knowing the thickness of the section (5μm), the mean length of the sperm head (115 μm, including the acrosome, N = 12) and the approximate length of the sperm-holding ducts, both the sperm density and

the total numbers of sperm present in the ducts could be estimated. This calculation assumes that the duct is full and isodiametric, the sperm orientation is random and the density homogeneous. A total of 252 sections of epididymides were examined.

7. Mean thickness of the wall of the epididymis. Ten randomly selected radial measurements were taken of wall thickness on each specimen. Care was taken to avoid measurements of oblique wall sections.

8. The volume of the seminiferous tubules as a proportion of the whole testis was calculated by measuring the proportion of seminiferous tubule against other tissues (capsule, blood vessels, lymph vessels and interstitial tissue) across ten random diameters of each testis. Estimates of the relative amounts of each component of testicular tissue were made to determine whether there was any differential development of the seminiferous tissues.

Four types of graph are presented.

1. Testis and ovarian follicle measurements were compared with SVLs to determine size at sexual maturity and to demonstrate ontogenetic allometry.

2. Collection dates were compared to SVLs to provide supporting evidence for activity seasons (most geckos are caught while active) and for iteroparity (if hatchlings are found in more than one season).

3. Left and right testes volumes were compared to determine whether sampling of only one gonad was appropriate.

4. Testis volumes and ovarian follicle measurements of adult geckos were compared with month of collection to show variation of reproductive activity with season.

Size at sexual maturity was determined from measurements of gonads, from histology of gonads and (and approximately confirmed from observations of live geckos). The size at sexual maturity is defined here as the minimum SVL at which there is an abrupt increase in the size of the gonads relative to SVL, when gametogenesis is assumed to begin. In eight of 18 cases, species samples were inadequate to determine an inflection point (males of *N. deleani, N. stellatus* and *N. wheeleri,* and females of *N. amyae, N. deleani, N. sheai,*

N. stellatus, and *N. vertebralis*), in which case estimates were made based on the mean % of maximum SVL values for the genus. For *U. sphyrurus* the estimate was based only on *U. milii*. Gonad measurement graphs of less than 12 points are not presented because of their limited value.

Description of the reproductive cycles is based largely on those species for which the most data are available (*N. laevissimus, N. levis* and *U. milii*) to which the limited data from other species were compared.

4.3 Results

4.3.1 Courtship and Mating

Adult specimens of *N. amyae, N. asper, N. levis, N. stellatus, N, wheeleri, U. milii* and *U. sphyrurus* were observed in captivity. I observed courtship on many occasions and mating behaviour in *N. asper* (once), *N. levis* (twice) and *U. milii* (three times). Mating in all three species was similar in basic characteristics. The pre-mating activities in *Nephrurus* and *Underwoodisaurus* geckos were variable and sometimes complex. The following is a composite account of courtship and mating in *U. milii* that were held in a large terrarium in early summer and at temperatures ranging from 22 - 28 °C.

Initially the male approaches the female from behind or from the side and sniffs and/or licks the flanks or pelvic area and occasionally the tail (a 'sniff' is defined as contact or close approximation by the tip of the snout but with no evidence of licking). Unreceptive females ignore the advances or move away. Receptive females usually remain stationary, turn their head towards the male and/or arch the tail. The male then moves out more or less parallel to the side of the female in short rapid movements and moves in front of the female with the head turned towards her. Sometimes these preliminaries may last only a minute or so, but he then approaches the female, puts one forelimb over her back and grabs a small pinch of the mid-vertebral skin of the posterior nuchal or shoulder region with the apical region of his jaws (Figure 4.2). The male then puts the corresponding hindlimb over the female's pelvis area and then positions his tail base under her tail base to bring the

ventrolateral aspects of their cloacae into apposition, possibly with the help of the cloacal spurs. Intromission follows almost immediately with only the hemipenis on the side adjacent to the female being everted. The female's tail is angled upwards from the base, arched with the tail tip off the substrate and twisted around the long axis through an angle varying from 20 - 80° to facilitate mating. The male's tail is also twisted through 40 - 60° in the opposite rotation, but remains more or less straight. The amount of arching and angulation is significantly reduced if one or both partners have a regenerated tail. The male maintains an uninterrupted grip on the skin of the shoulder area throughout the mating and makes frequent intermittent twitches of the head and body. Each twitch consists of a short vibration or shudder of low amplitude, but sufficient to induce a slight passive shudder in the female. The pair remain conjoined at the cloacae for over half an hour with the female remaining stationary or occasionally walking around (possibly because of disturbance by observer). The male maintains his grip on the skin for about a minute even after genital separation. Hemipenis detumescence almost certainly begins prior to genital separation, but requires approximately another 10 minutes for complete retraction. The male gecko keeps the cloacal region well off the substrate and frequently licks, nibbles and tugs at the portion of the hemipenis still exposed. Licking of the cloacal area continues intermittently for several minutes, even after complete retraction of the hemipenis.

On one occasion, a female *U. milii* that had mated less than one hour before was approached by a second male when she adopted the arched tail attitude typical of receptive females but, apart from a lick from the male, there was no further interaction. Several captive female *U. milii* engaged in courting behaviour one day after egg laying and one mating was observed the night after egg laying.

Similar or more complex behaviours were seen in *Nephrurus* geckos. For example an adult female *N. levis* walked around the male with head down, legs partly flexed, pelvis raised off the substrate and the tail gently arched, a behaviour that seemed to indicate sexual receptivity (see below). A complex interaction between a male and female *N. asper* involving the caudal knob was also observed. A male *N. asper* that was courting a female was running jerkily around the female which, when he approached her closely, immediately turned away and vibrated her large tail knob very rapidly in his face for several seconds so that it repeatedly touched many points around his snout, as if 'feather-dusting' his face. The

male remained motionless during this procedure and for several seconds afterwards. The female moved away, the male did not follow and did not resume courtship behaviour. It is possible that the female was already gravid at the time, as the pair had been maintained together for several months.

One adult male *N. asper* spent approximately 30 minutes running jerkily around an adult female, which seemed to take little notice and continued wandering around the enclosure. When another gecko (a female *N. levis*) came between them, the male briefly followed and vigorously courted this second gecko, but after several seconds seemed to realise his mistake (without closely approaching the female *N. levis*) and went back to courting the female *N. asper*. Male *N. asper* makes repeated soft chirping noises while mating. During courting and mating in *N. asper* and *N. levis*, the tail knobs are intermittently waved from side to side by both males and females. During courting the male often drags his oscillating caudal knob along the substrate leaving a wavy line in the sand.

The duration of the period from fertilisation to egg laying is difficult to determine. A minimum of 19 days between clutches has been recorded for *N. asper* in a heated vivarium (H.-J. Sameit, personal communication). One specimen of *U. milii* kept at an average room temperature of approximately 25 °C laid a clutch of eggs 29 days after mating. No female of any *Nephrurus* or *Underwoodisaurus* species was found with both oviductal eggs and fully mature ovarian follicles.

Underwoodisaurus milii can live at least 16 years and *N. levis* at least 6 years (personal observations), with reproduction occurring throughout life with the probable exceptions of the first and last one to three years (assuming death from 'old age').

4.3.2 Analysis of Museum Specimens

4.3.2.1 Male Reproductive System

Apart from size, the testes are very similar in all study species and are therefore considered together. They are paired, cream coloured (pale pink in life) organs located in a posterior dorsolateral position and each is supported by a longitudinal fold of peritoneum, the mesorchium (Figure 4.3). Each testis is supplied by a short renal artery branching from the

aorta and drained by a renal vein attached to the posterior vena cava; both vessels are supported by the suspensory mesorchium. The position of the testes varies little, being situated close to the spinal column, overlapping but slightly anteroinferior to the kidneys. The two testes often are slightly offset longitudinally with the right one being more anterior. A thin fibrous connective tissue capsule, the tunica albuginea, covers the testis and beneath the capsule is an extensive vascular layer, the tunica vasculosa (Figure 4.4). The major part of each testis consists of the seminiferous tubules, each of which is covered by a thin fibrous capsule (tunica albuginea). Blood and lymphatic vessels, as well as interstitial cells, occupy the spaces between the seminiferous tubules. Attached to each testis is a system of (Wolffian) ducts composed of the vasa (or ductuli) efferentia, the epididymis and the vas deferens, which opens into the cloaca. Branching just proximal to the origin of the epididymis produces a variable number of vasa (or ductuli) efferentia in lizards (*e.g.*, six in *Scincus* and one in *Lacerta*, Fox, 1977). The vasa efferentia emerge from the testicular capsule and then branch to form a reticulum with numerous branches, some of which are blind ended, and others eventually fuse to form a single epididymis and then vas deferens duct.

At hatching, the testes are very small and inactive (Figure 4.5). All species (where adequate data are available) show approximately isometric testis growth with respect to SVL). However, at a certain stage of development they all show a large increase in growth rate signifying sexual maturity. Testis growth in the larger adults varies little among species ranging from close to isometric (*e.g.* in *N. laevissimus*) to positively allometric with respect to SVL (*e.g.* in *U. milii*). The adult tubules are turgid beneath the connective tissue capsule, the tunica albuginea, when in breeding condition. The adult quiescent testis is not swollen and the convolutions of the seminiferous tubules are less distinct.

The testis of juvenile *U. milii* (Figure 4.5) has fewer seminiferous tubules and is less vascular compared to the testis of adults. The juvenile seminiferous tubules are relatively smaller in diameter than in the adult and have fewer cells, particularly spermatogonia, in the wall (compare Figure 4.6, 4.5). There is also little evidence of immature seminiferous tubule sections compared to the active adult testis (Figure 4.6). Scale bar = 0.5 mm.

Figure 4.2 *Underwoodisaurus milii* showing mating position adopted by both *Underwoodisaurus* and *Nephrurus* species.

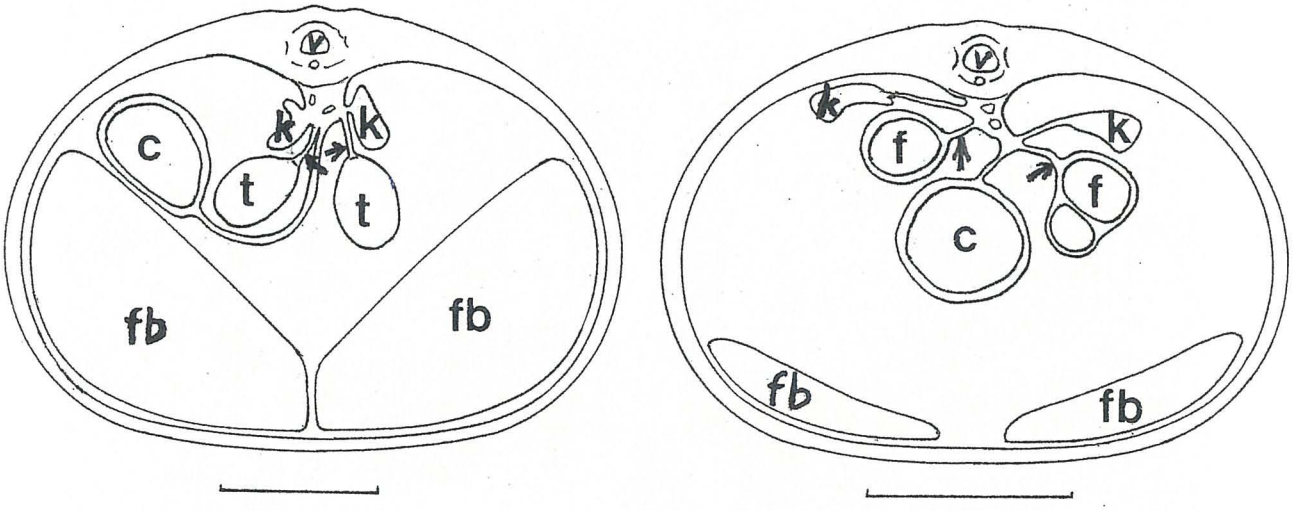

Figure 4.3 Cross section of body of adult male *U. milii* (TA1760, left) and adult female *N. levis* (TA1215, right) showing relationship of gonads to serosa and body wall, contiguous surfaces have been slightly separated to clarify outlines (loops of small intestine not shown). t = testis, f = ovarian follicle, fb = fat body, k = kidney, arrow = mesovarium in female and mesorchium in male.

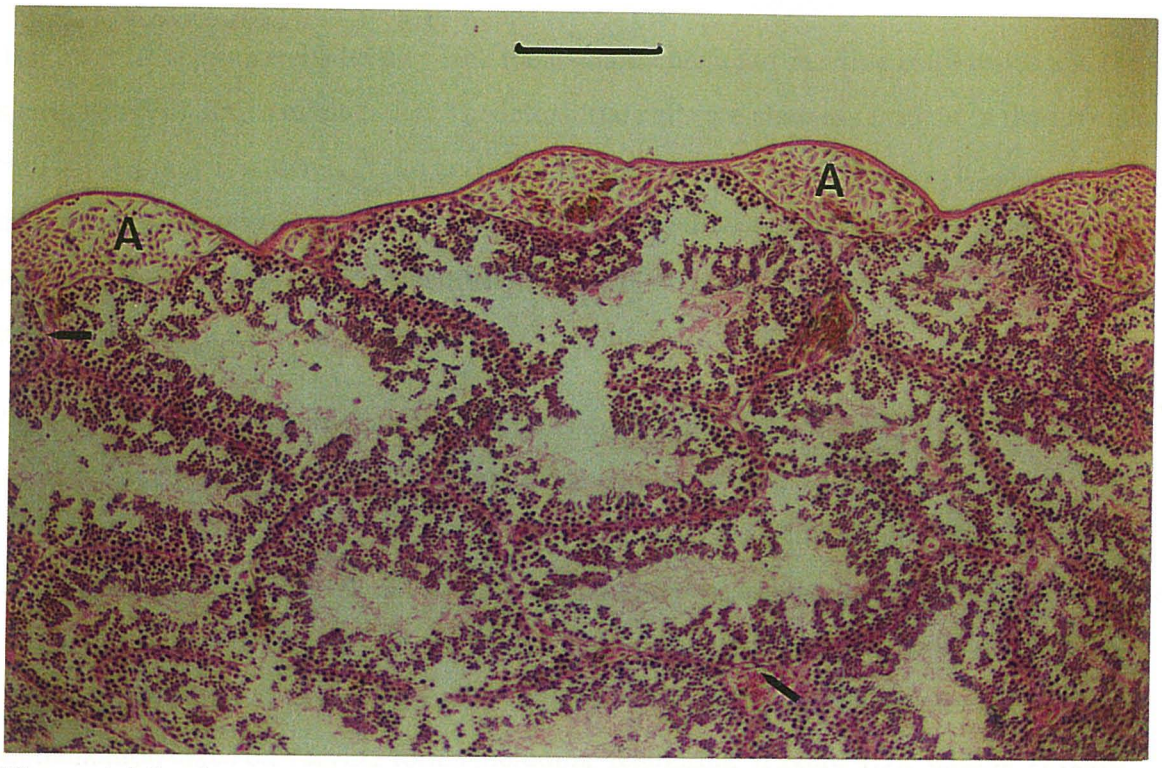

Figure 4.4 Section through testis of adult *N. levis* R96130, collected in October, showing tunica vasculosa (A) and capillary extensions between seminiferous tubules (arrows). Scale bar = 0.2 mm.

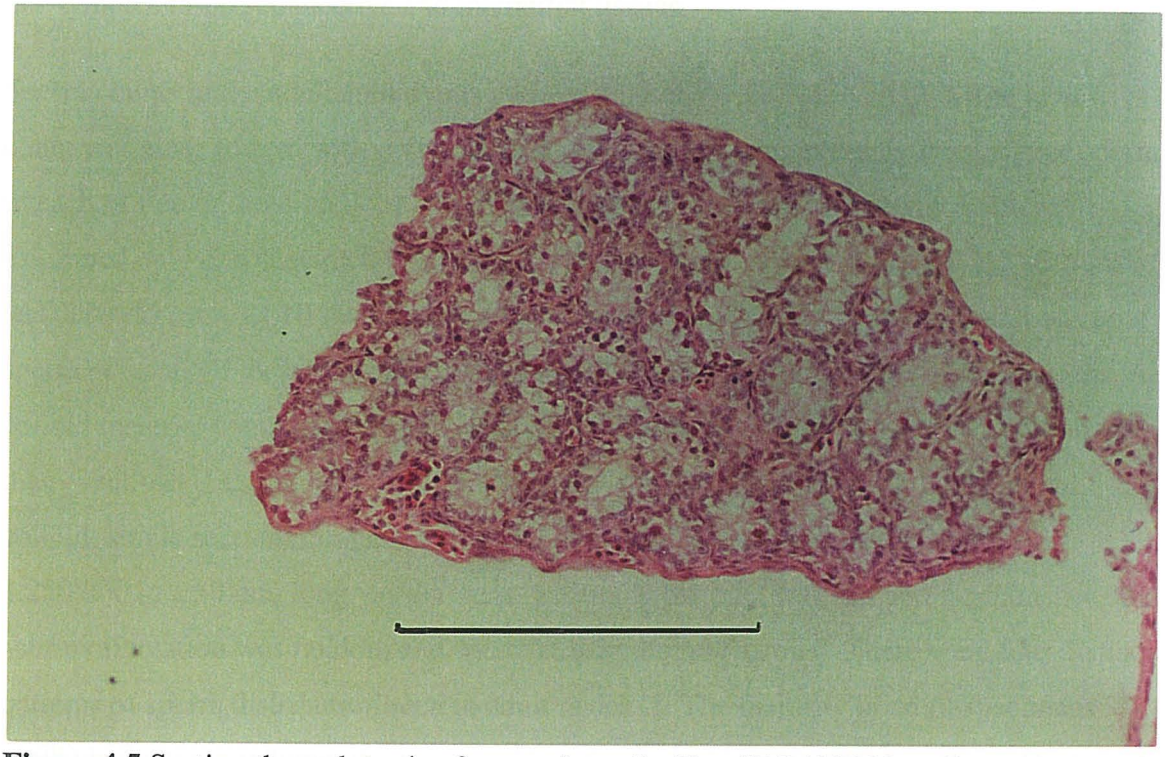

Figure 4.5 Section through testis of young juvenile *U. milii* R129328, collected in January, showing a thin enveloping tunica albuginea, reduced vascularity and reduced number of specialisation or cytoplasmic activity with a less granular cytoplasm and few mitotic figures.

The juvenile seminiferous tubules lack the pycnotic nuclei of inactive adult testes and the sperm heads of active adult testes. The interstitial cells are also less evident in the juvenile testis. The point at which a gecko becomes sexually mature cannot always be precisely determined in terms of SVL. For example, the smallest *N. asper* male with enlarged testes was 63 mm SVL, but another specimen of 65 mm SVL had few sperm developing in the testis and none in the vasa efferentia or epididymis.

Adult testes are variable in their level of activity and histological appearance. Some adult testes show very low activity, for example one specimen of *N. wheeleri* collected in June had no sperm or spermatids present (Figure 4.11). All other adult testes showed some sperm production activity. Some sections of seminiferous tubules show segmentation of the internal surface with a variety of 'clumps'. This segmentation effect is obvious in some longitudinal (Figure 4.7) and transverse sections (Figure 4.8), but not in others. The segmentation takes on varying forms including 'feathering' (Figure 4.9), 'tenting' (Figure 4.10) and clumping (Figure 4.7). Most of the preserved adult testis volume is occupied by convoluted seminiferous tubules (83.7 ± 9.3%, N = 10 *N. levis*), the remainder being blood and lymph vessels, connective and interstitial tissues.

Sperm counts in the adult epididymis and vas deferens were very variable. The lowest count was close to zero with no sperm in most of the sections and only two or three sperm in each of two or three sections. Of 20 specimens of adult epididymis/vas deferens examined only one specimen (5%) had no sperm present (*N. wheeleri*). Of 252 sections of vas deferens (at least 10 sections per specimen) examined, 200 (79.4%) had at least some sperm present. Of these, 73.5% had high-density sperm present in most of the sections. A colloid material containing protein (as shown by staining pink with eosin) was found in many sections (Figures 4.11, 4.16, 4,17). When the vas deferens was full of seminal colloid, single sections contained up to 1,250 sperm heads at a density calculated at 1,250,000 in a 50 mm long vas deferens (assuming the duct was full and isodiametric, that sperm orientation was random and sperm density homogeneous). There were four distinct patterns of sperm distribution seen in adult males. 1. The majority of adult specimens (75%) had a large volume of

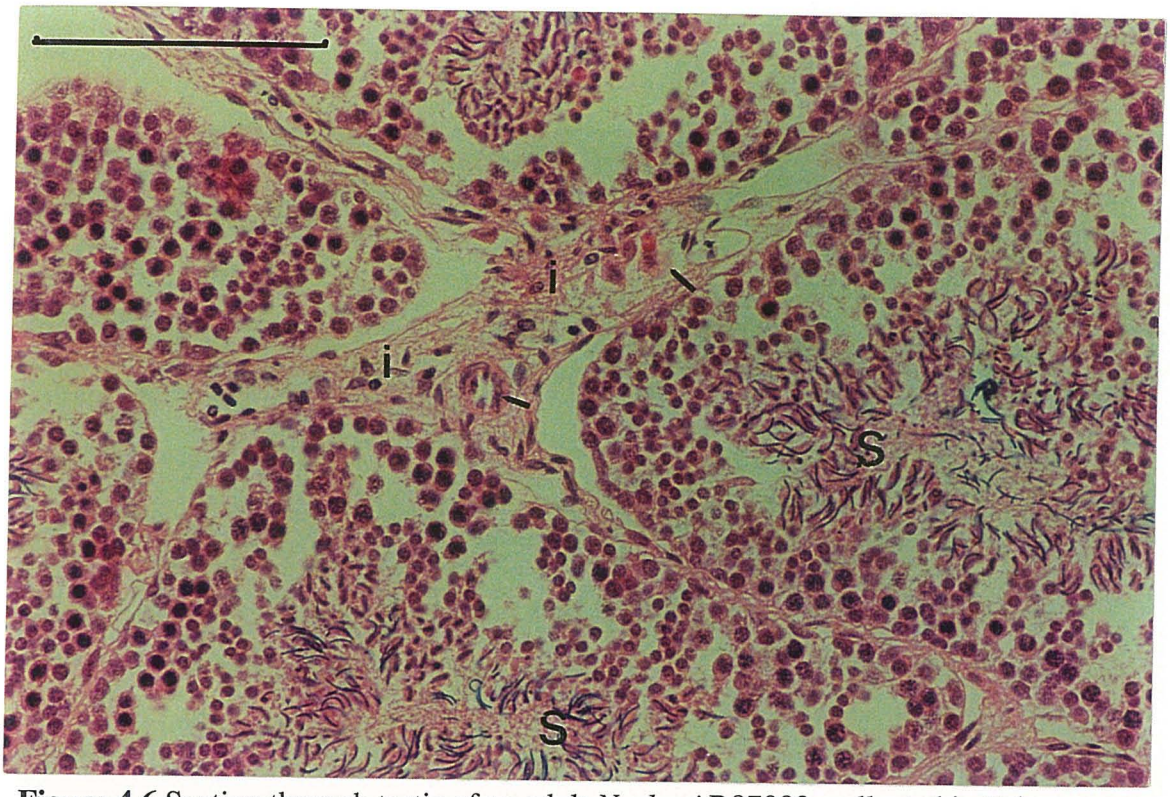

Figure 4.6 Section through testis of an adult *N. sheai* R87082, collected in February, showing blood vessels (arrows), interstitial cells (i) and active seminiferous tubules with developing sperm (S). Scale bar = 0.1 mm.

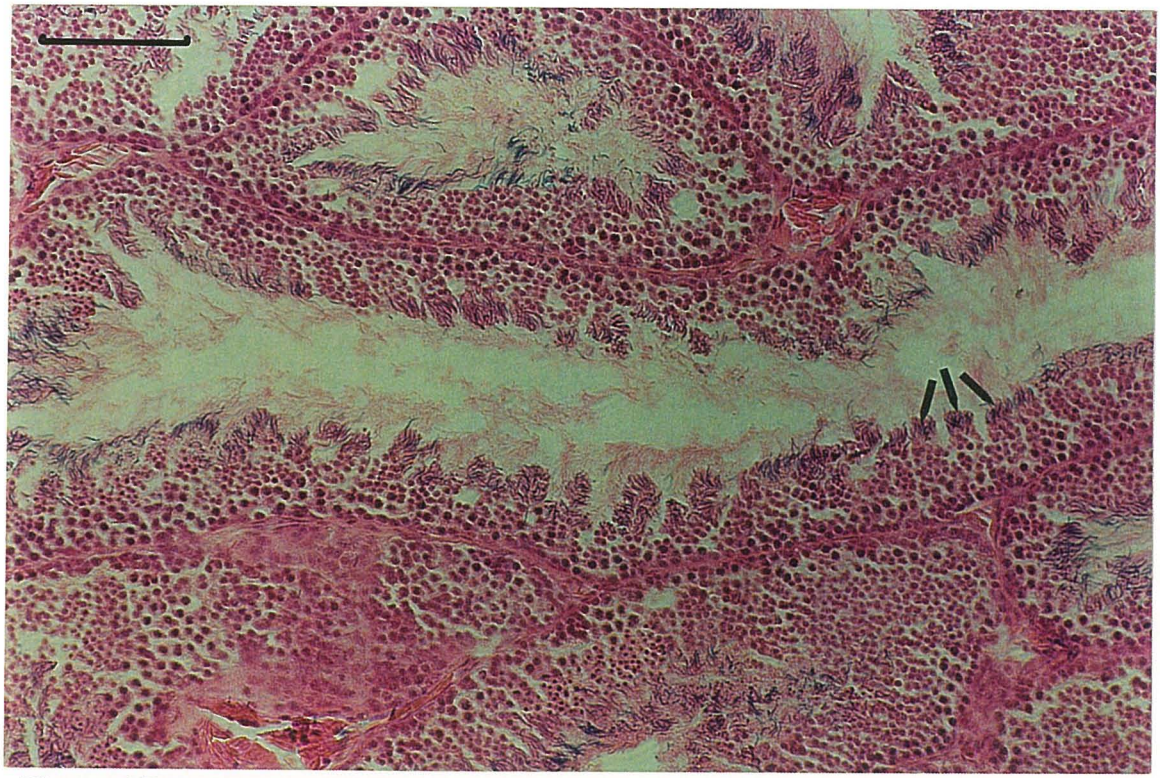

Figure 4.7 Section through testis of adult *N. levis* R20353, collected in July, showing active spermatogenesis and 'clumping' formation of spermatogenous clones (arrows). Scale bar = 0.1 mm.

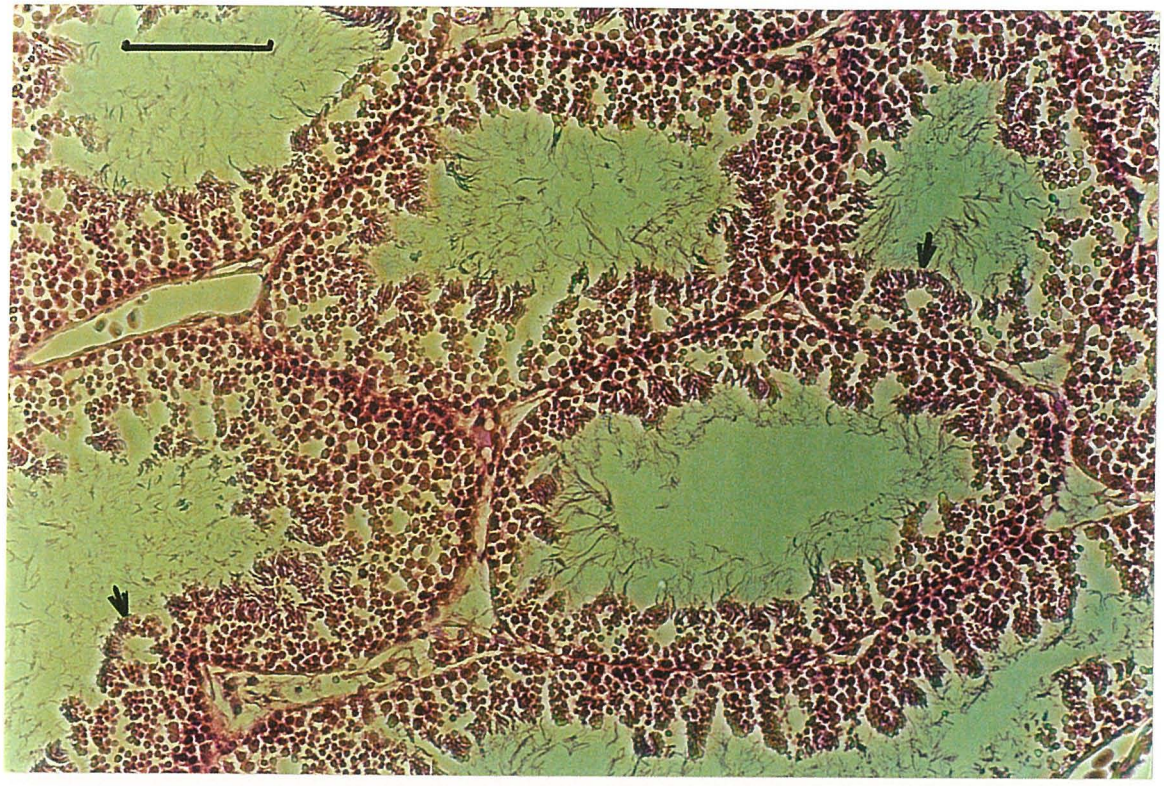

Figure 4.8 Section through testis of adult *N. levis* R69205, collected in May, showing active spermatogenesis with 'clumping' and 'tenting' (arrows) of spermatogenous clones. Scale bar = 0.1 mm.

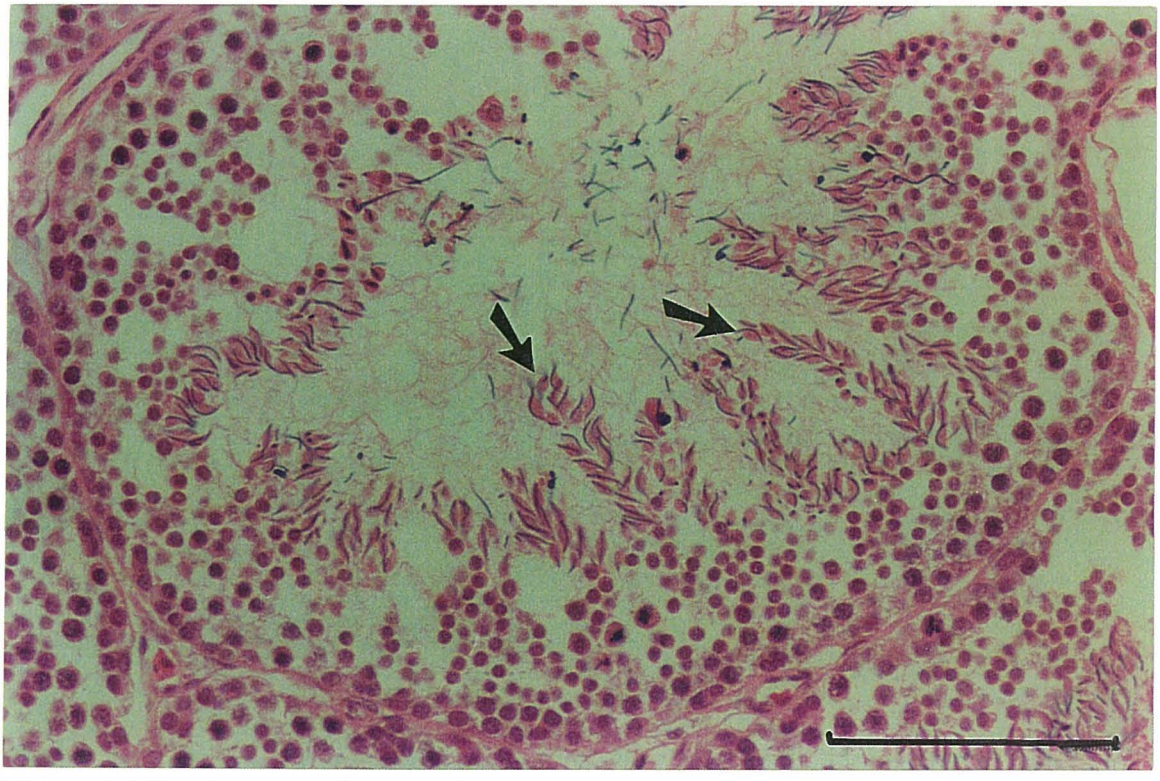

Figure 4.9 Section through testis of adult *N. laevissimus* R72738, collected in October, showing 'feathering' formations (arrows). Scale bar = 0.1 mm.

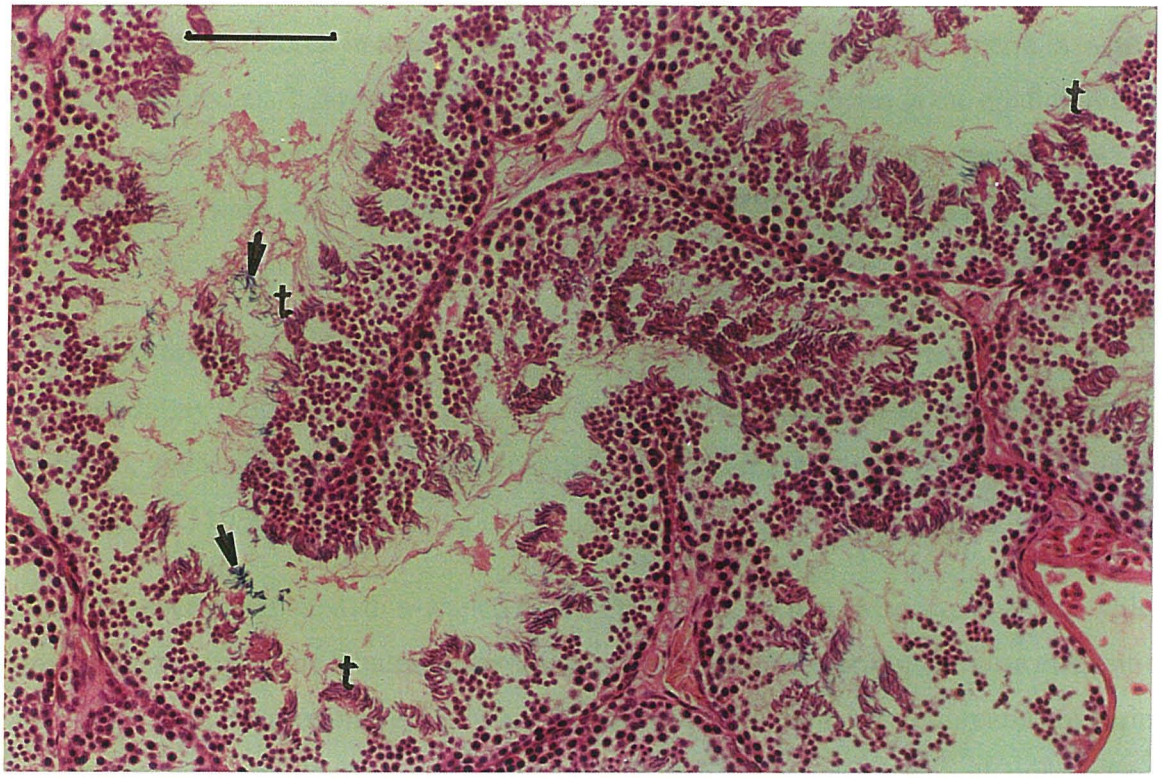

Figure 4.10 Section through testis of adult *N. levis* R110594, collected in November, showing 'tenting' formations (t) and low rate of spermatogenesis with few sperm (arrows). Scale bar = 0.1 mm.

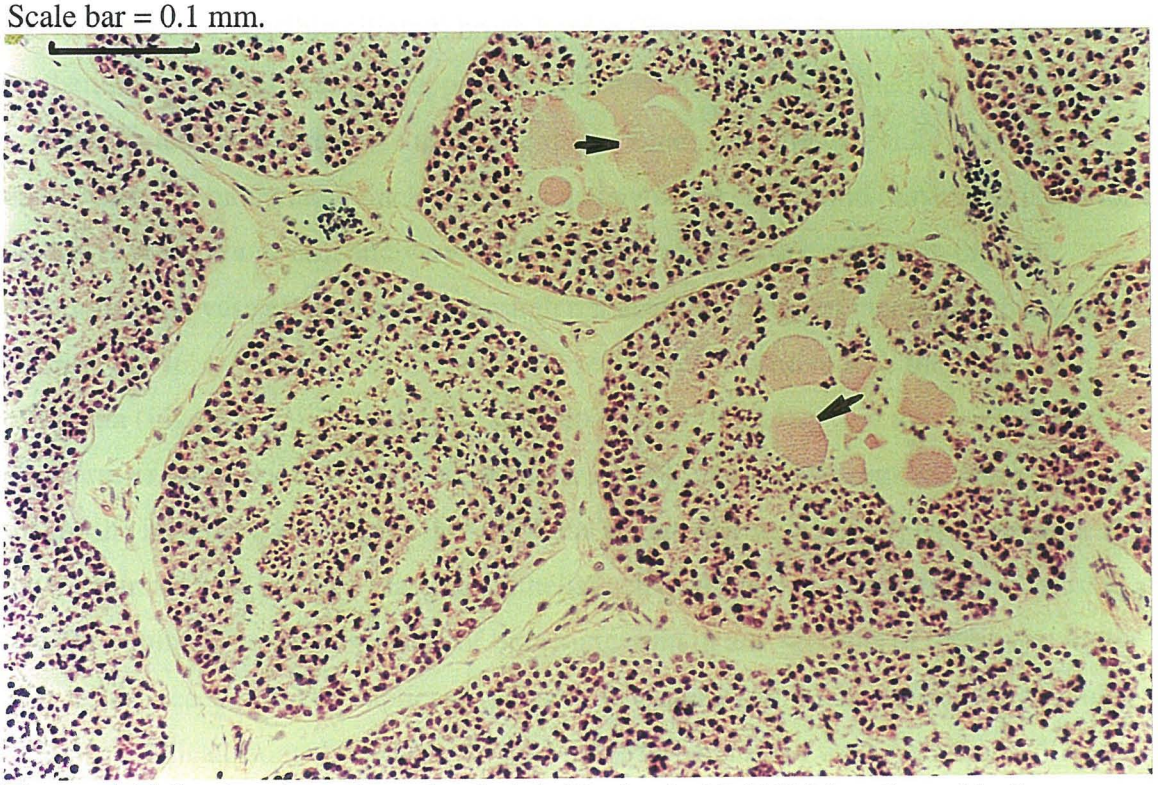

Figure 4.11 Section through testis of adult *N. wheeleri* R125024, collected in June, showing inactive seminiferous tubules with no spermatids present and the accumulation of numerous cells and colloid material (arrows) in lumina. Scale bar = 0.1 mm.

high-density sperm stored in the epididymis and vas deferens with a lot of sperm in active seminiferous tubules. 2. Two specimens had a large number of sperm in the epididymis, but very little activity in the testis. 3. In one specimen, the spermatogenesis appeared very active but there were very few sperm in the epididymis. 4. Two specimens had few sperm in both the epididymis and the testes.

The spermatozoa of both *N. levis* and *U. milii* are similar with long thin heads, correspondingly long thin acrosomes and mid-pieces as described for squamate reptiles (Jamieson, 1995). The maximum total length of an *U. milii* sperm is 475 μm and the mean head length is 115 ± 6 μm, including the acrosome (N = 12).

There are several branched, thin-walled ducts, the vasa efferentia (MacAvoy, 1976), which connect the seminiferous tubules to the single epididymis. The loops of the vasa efferentia intermingle with the proximal epididymis (Figures 4.12, 13). The vasa efferentia are varied in diameter and the walls consist of an outer connective tissue layer, a smooth muscle layer and an inner simple cuboidal (in some cases almost squamous) epithelium with a luminal stereociliated (or possibly ciliated) brush border (Figure 4.13). In presumed active areas the epithelium becomes thickened, appearing columnar or pseudostratified. The vasa efferentia are convoluted and anastomose to form a single, much convoluted epididymis. The epididymis is a moderately thick-walled and convoluted duct found dorsal and posterior to the testis. Very few sperm and very little secretory material are present in the vasa efferentia. The vasa efferentia pass through a transitional stage of increasing diameter to become the epididymis, which has a thicker secretory/absorptive wall than the vasa efferentia (Figure 4.13). The inactive adult epididymis has relatively thin walled ducts and no sperm or secretory material present (Figure 4.14). The juvenile epididymis is similar to that of the inactive adult, except that the ducts are smaller in diameter, less convoluted and have a thinner wall lined by inactive epithelial cells with less secretory material present and smaller lumina. The only evidence of branching seen in any sections of testes was in the 'rete testis' area, where the duplicated sections of the efferent ducts just below the tunica albuginea indicate some branching. In the vasa efferentia there were occasional 'Y' shaped ducts, indicating that some branching occurs in this area. No evidence of branching was seen in the epididymides.

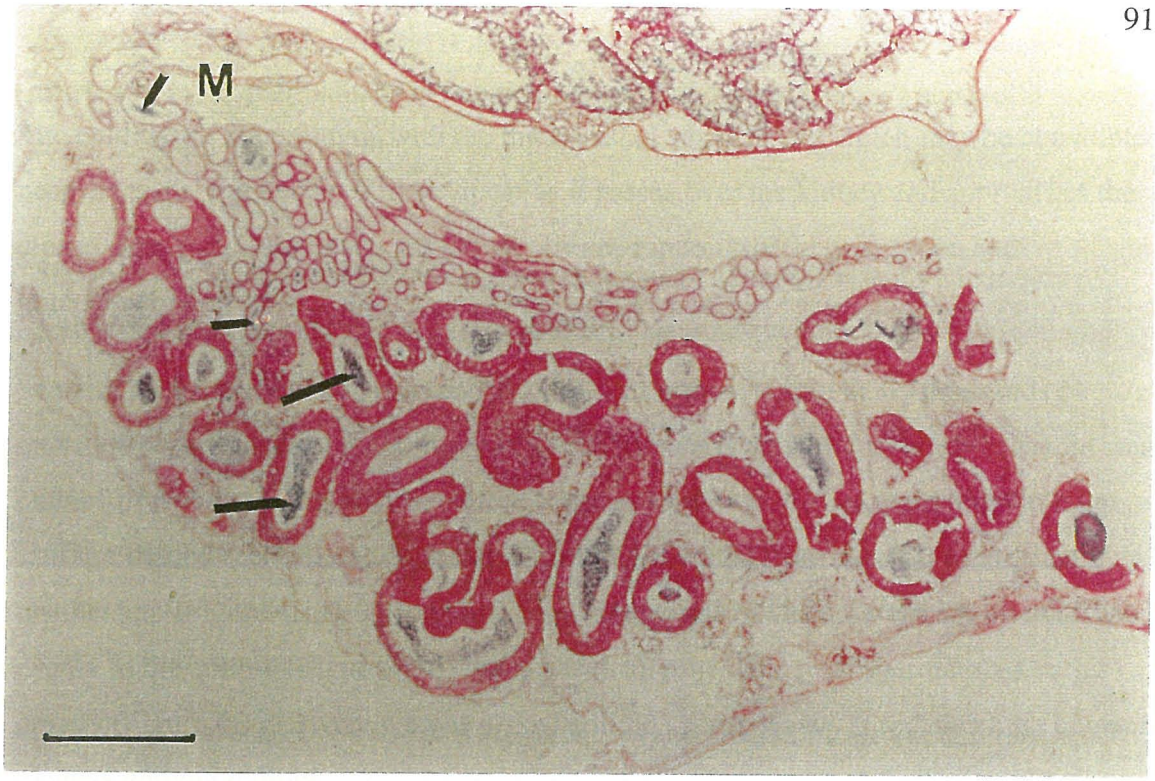

Figure 4.12 Section through active epididymis of adult *N. asper* R110562, collected in November, showing mesorchium (M), which is continuous with the capsule of the epididymis. Several of the vasa efferentia contain sperm (small arrows) and most of the sections of the epididymis also contain sperm (large arrows). Mean epididymis wall thickness = 47 μm ± 1.0 (N = 18 points). Scale bar = 0.5 mm.

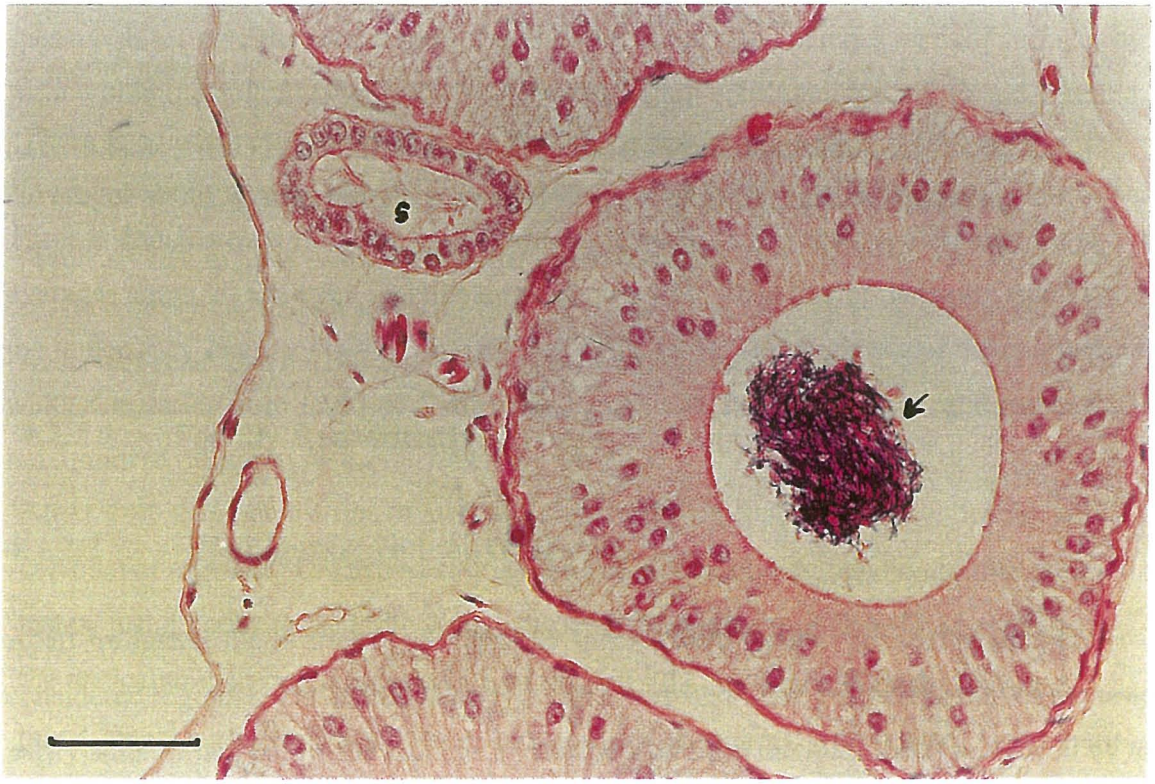

Figure 4.13 Section through active epididymis of adult *N. laevissimus* R86002, collected in February, showing much-thickened wall of pseudostratified epithelium and dissolving colloid with high sperm density (arrow). Mean wall thickness 61.6 μm ± 9.1 (N = 10 points). Note stereocilia in vasa efferentia (s). Scale bar = 0.1 mm.

The epididymis is continuos with the thick walled vas deferens which may be convoluted near the testis, but becomes more linear as it passes over the kidney and approaches the cloaca. Many sections of the vas deferens show random sperm orientation and homogeneous sperm density.

Sections of the adult epididymis and vas deferens either contain many thousands of embedded spermatozoa or are empty of sperm, or nearly so with just a few scattered small clumps of isolated gametes. The colloid contents of the vas deferens are often in long sausage shaped sections and each bolus is often separated from the wall of the duct by a distinct gap (Figure 4.15). The sperm of the epididymis or vas deferens are often distributed evenly in the colloid (Figure 4.16) but occasionally the density of spermatozoa varies greatly (Figure 4.17). Even in areas of low colloid sperm density it remains high compared to areas without colloid.

Occasional cross sections towards the middle of the epididymis or vas deferens are devoid of colloid and spermatozoa, indicating that the colloid/sperm bolus is not always continuous throughout the duct (Figure 4.12). In some parts of the vas deferens the colloid/ sperm boluses give the appearance of dissolving in localised patches on their surfaces or from within the colloid (Figure 4.13). Almost all adult epididymides and vasa deferentia have at least a few spermatozoa and some colloid material present.

There is considerable variation in the luminal diameter of the adult epididymis, which averages about 90 μm in an inactive duct (Figure 4.14). In active or distended epididymides the lumen may range up to 420 μm (Figures 4.13, 4.16). The thickness of the epididymis walls also varies from 10.5 - 47.0 μm (N = 10 sections, in adult *N. levis*) and reaches a maximum of 56 μm in *N. laevissimus* (Figure 4.13).

4.3.2.2 Female Reproductive System

Apart from size, the ovaries of all the study species were similar; hence they are here considered together. The paired ovaries are in a corresponding position to that of the testes, partly anterior and below the kidneys and suspended from the mid-dorsal body wall by the membranous mesovarium (Figure 4.3). In occasional specimens, they are more deeply seated in the pelvis *i.e.*, more caudad, compared with others. At hatching,

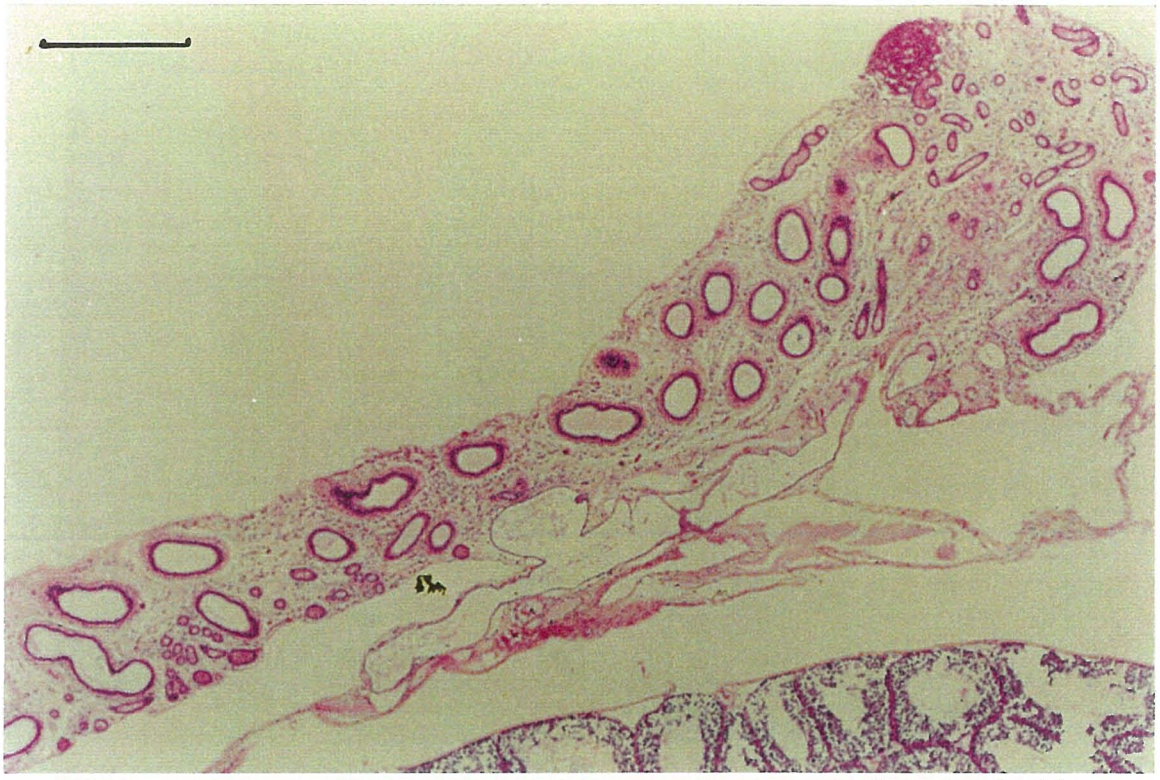

Figure 4.14 Section through inactive adult epididymis and vasa efferentia of adult *N. wheeleri* R102163 collected in August showing thin walls and lack of secretory material. There are also no sperm in the seminiferous tubules. Scale bar = 0.5 mm.

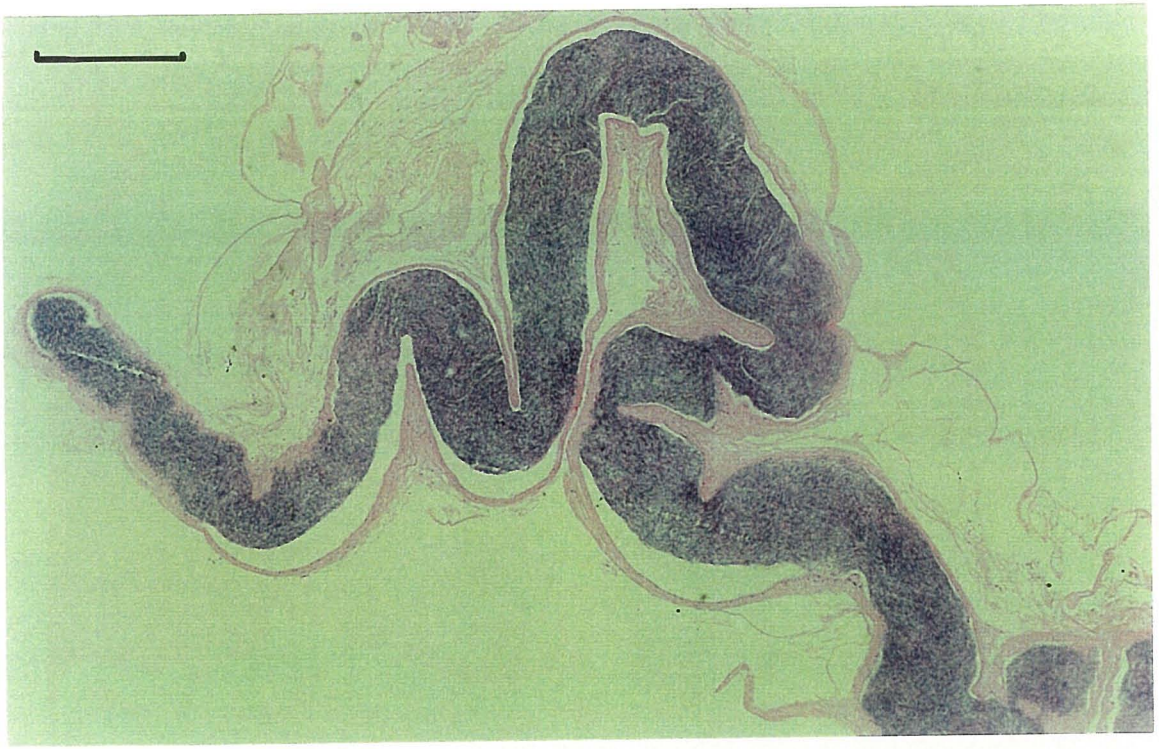

Figure 4.15 Longitudinal section through proximal vas deferens of adult *N. sheai* R87082, collected in February, showing storage of a large number of sperm and possibly artifactual variation in gap between sperm bolus and wall. Scale bar = 0.5 mm.

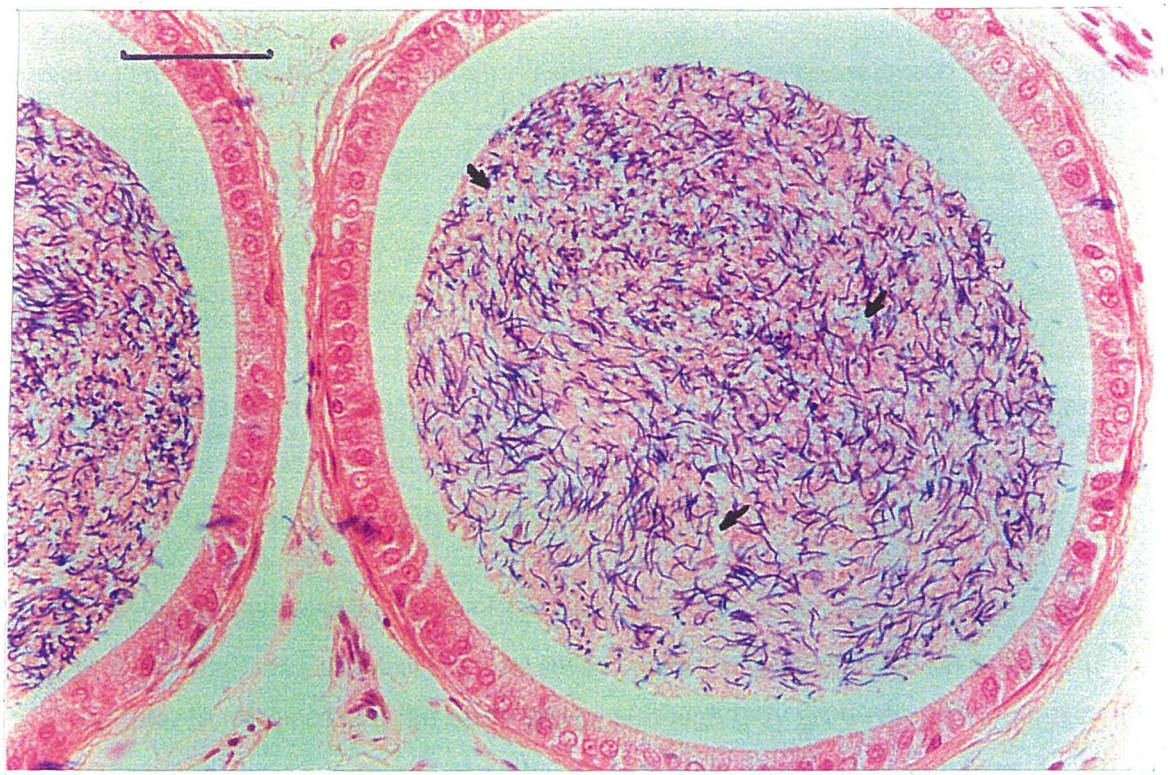

Figure 4.16 Section through epididymis of adult *N. sheai* R87082, collected in February, showing cuboidal epithelium, dense sperm cells and numerous pale spots (arrows) representing dissolution of colloid. Scale bar = 0.1 mm.

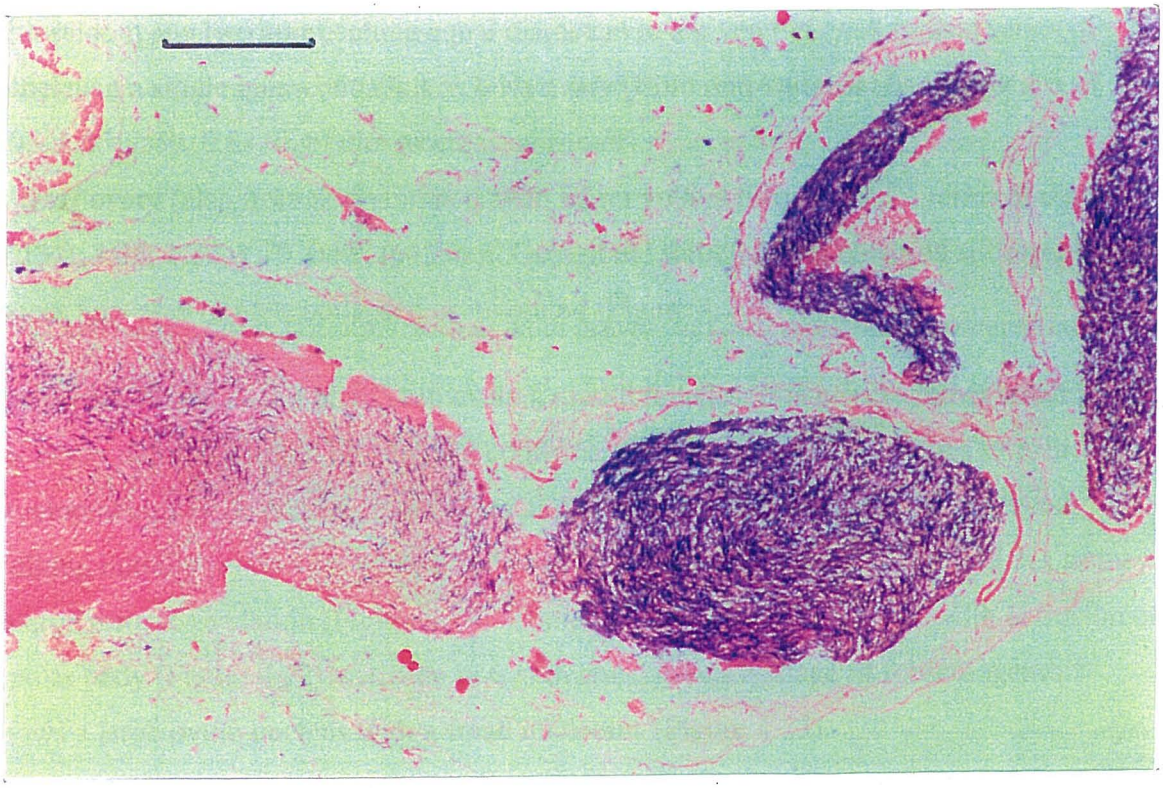

Figure 4.17 Longitudinal section through vas deferens of adult *N. sheai* R87082, collected in October showing low sperm density (left) and high sperm density (right) in colloid. Scale bar = 0.2 mm.

the ovaries are very small, but are usually distinguishable from testes at x40 magnification by the presence of one or two minute previtellogenic ovarian follicles, each measuring about 0.2 - 0.4 mm in diameter. Except in young juveniles, the ovary is small compared to the size of the follicles it produces. The volume of the hatchling's ovary (measured in preserved specimens of *N. amyae*, *N. asper*, *N. laevissimus*, *N. levis*, *N. wheeleri* and *U. milii*) ranges from 0.031μL in *U. milii* to 0.694 μL in *N. amyae*. There is limited further development of the ovarian follicles until the gecko approaches maturity. The follicles do, however, increase in size and number in the juvenile period so that the ovary usually becomes macroscopically distinguishable from the testis within days of hatching.

The adult ovary contains very little stroma and a small area of germinal epithelium that gives rise to the oocytes (Figure 4.18). The range of ovarian follicle sizes present in an ovary is similar in all species of the study group, although they grow absolutely largest in the larger species. The adult ovary normally has a variable number (ranging from 4 - 10) of very small to large follicles in various stages of yolking (Figure 4.19). Each follicle is enclosed within a follicular capsule (follicular epithelium, zonal pellucida and thecae) as well as the tunica albuginea. The ovarian follicles are invariably in a developmental series with seldom any two approaching equal diameters in a given ovary. A typical series of follicles in a single quiescent adult *N. levis* ovary (from mid-summer) had diameters of 2.06, 1.55, 1.50, 0.84, 0.40 mm whereas an active
N. levis ovary (also from mid-summer) with larger yolked follicles had a series of diameters of 13.2, 4.25, 2.78, 1.79, 0.89, 0.45 mm. Occasional follicles with enlarged subcapsular spaces appeared to have collapsed or involuted (Figure 4.20).

The corpus luteum is not often seen even in gravid females, it is usually rather small and not easily defined without histological confirmation (Figure 4.21). All stages of follicular development may be present in the ovary simultaneously during the breeding season. Yolking initially produces a whitish followed by yellow colouration of the follicles as they enlarge prior to ovulation (Figure 4.19). Completion of a meiotic division with extrusion of a polar body (Figure 4.22) was seen in two ovaries. An early stage of folliculogenesis shows a large ovum nucleus with a small nucleolus (Figure 4.23).

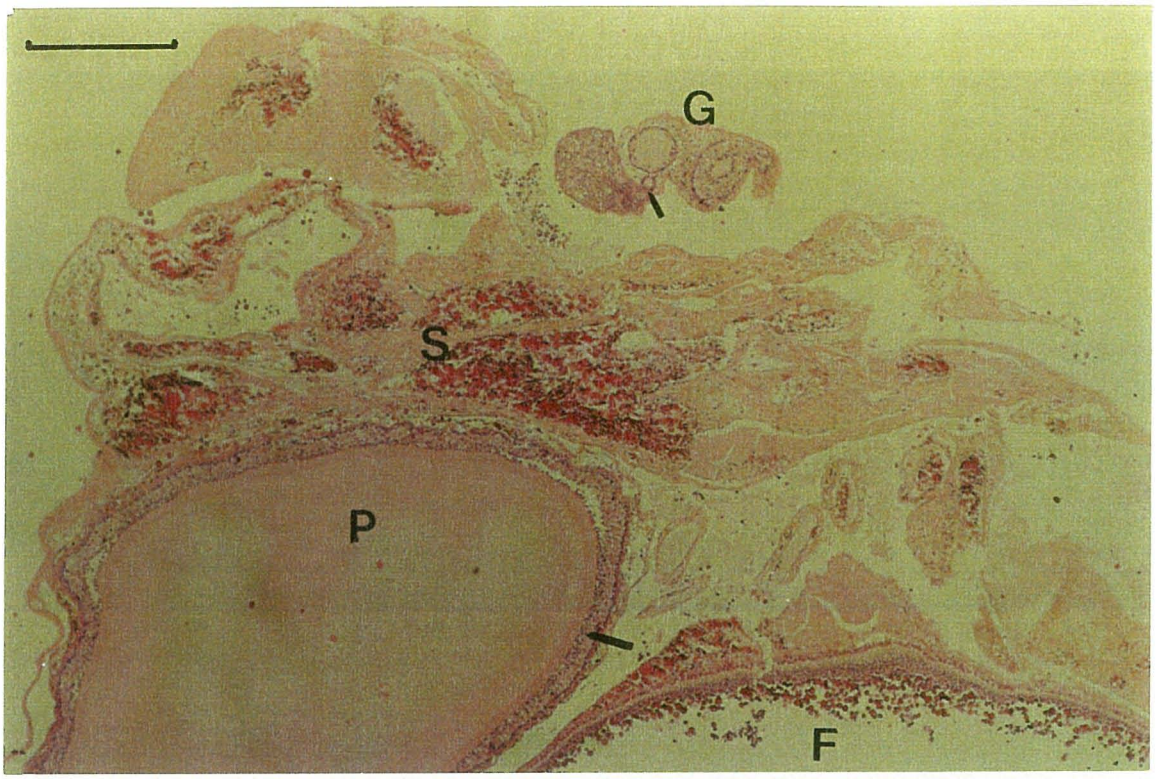

Figure 4.18 Section through ovary of adult *N. levis* R102603, collected in October, showing several stages of folliculogenesis, germinal centre (G), stroma with remains of atretic follicles (S), oogonium (short arrow), growing previtellogenic follicle (P), mature yolked, preovulatory follicle (F). The theca interna (long arrow) generates the yolk material and secretes oestradiol (Guraya, 1989). Scale bar = 0.5 mm.

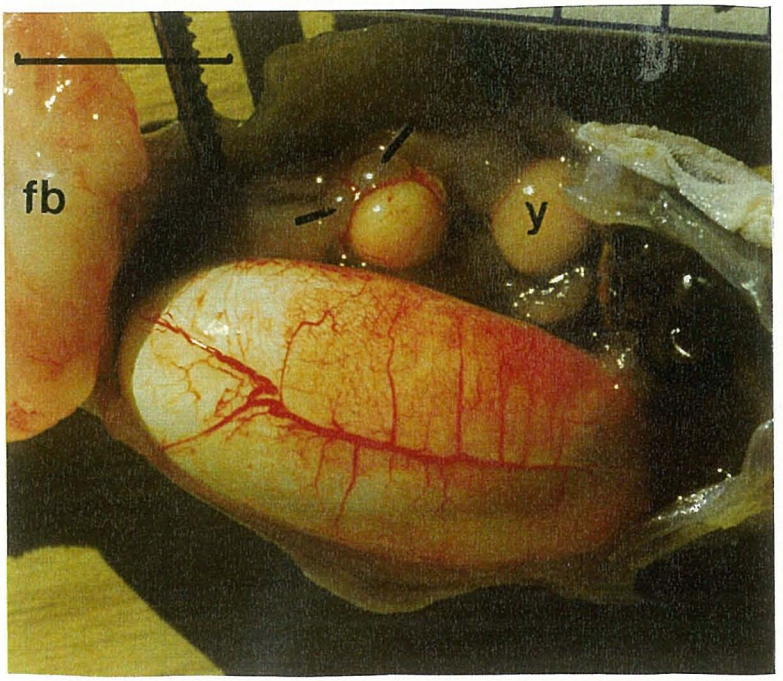

Figure 4.19 Yolked (y) and unyolked (arrow) follicles in ovaries of *U. milii*, TA1346, euthanased in January, shows large reflected fat body (fb) and an oviductal egg in well vascularized oviduct (both left and right ovaries visible). Scale bar = 10 mm.

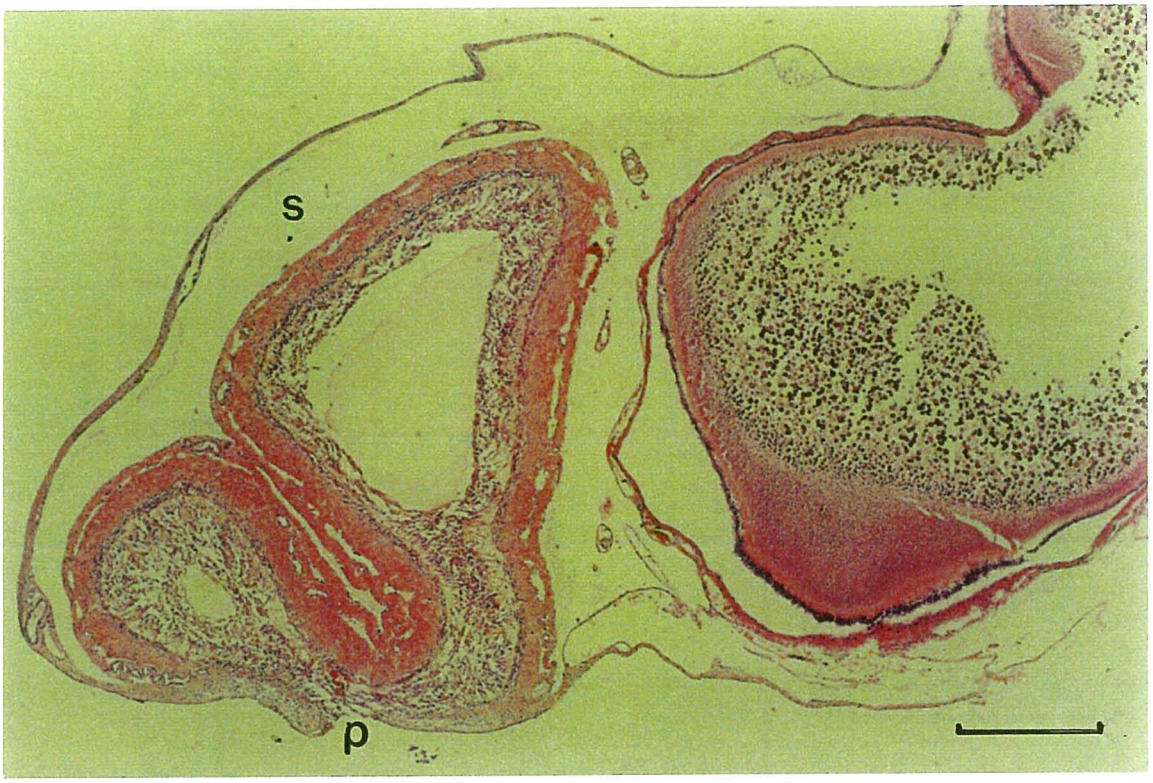

Figure 4.20 Section through ovary of adult *U. milii* R133978, collected in November, showing two involuting follicles with large subcapsular space (S) and infolding of follicle membranes. A rupture point is shown (p). Scale bar = 0.5 mm.

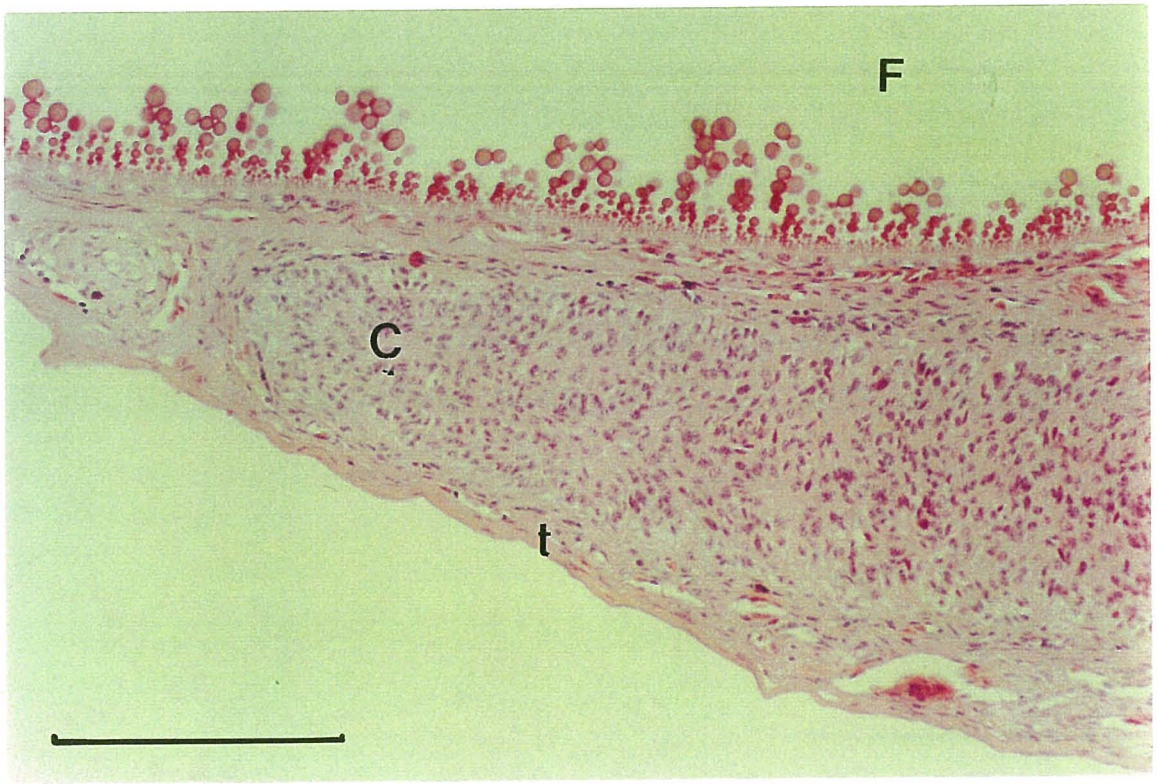

Figure 4.21 Section of ovary of adult *N. levis*, 855, collected in January, showing corpus luteum (C) sandwiched between the follicular thecae of a mature follicle (F) and the tunica albuginea (t) below. Scale bar = 0.1 mm.

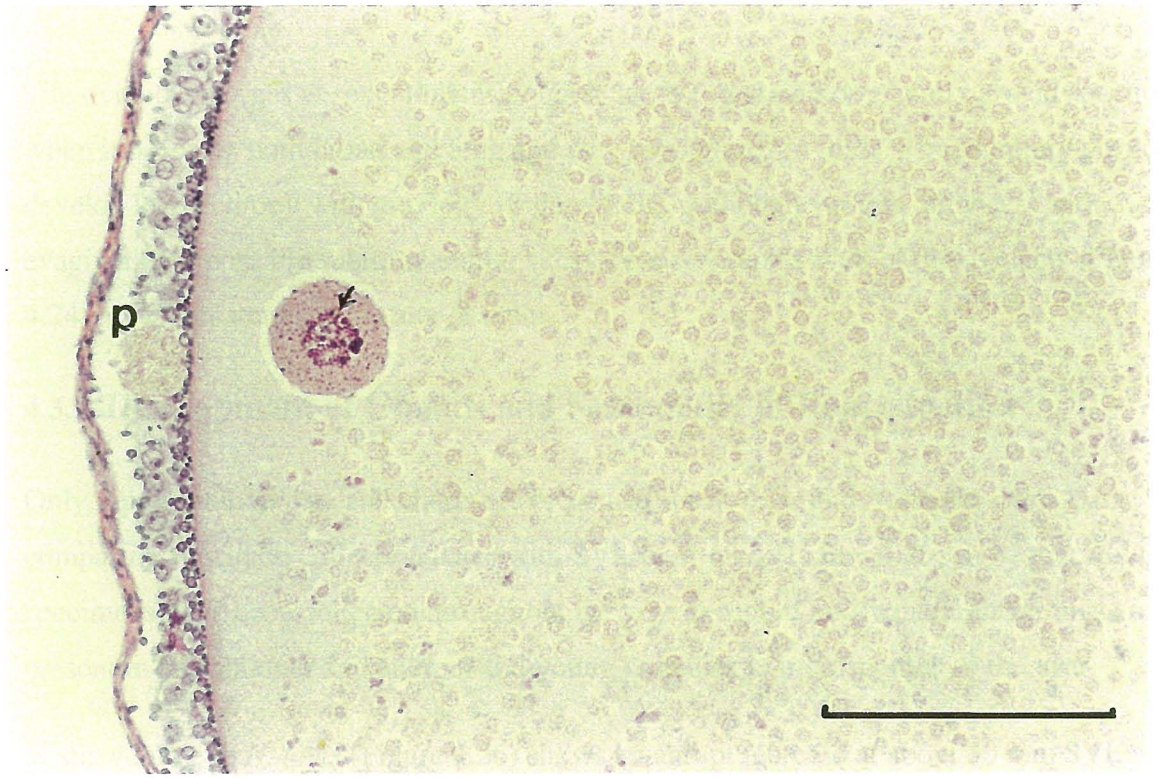

Figure 4.22 Section through ovary of adult *N. levis* (TA162), collected in December, showing part of mature yolked preovulatory follicle with enlarged nucleolus (arrow) in germinal vesicle and first polar body (p). Scale bar = 0.1 mm.

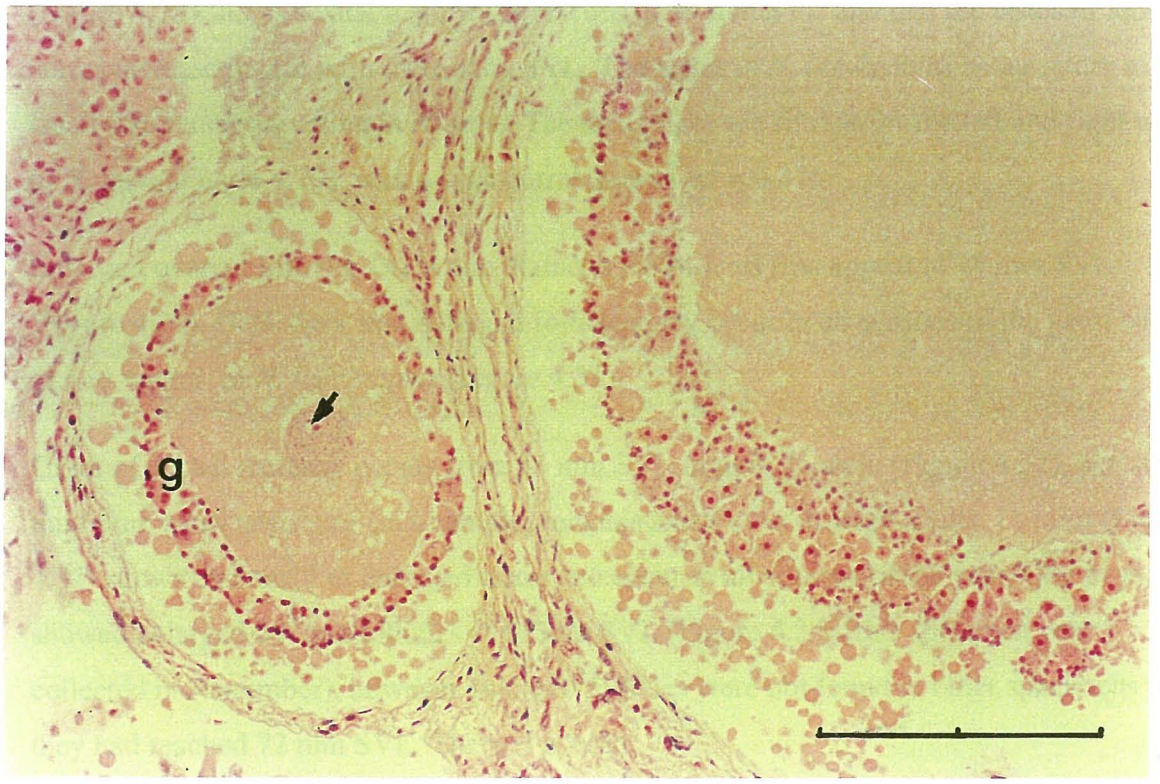

Figure 4.23 Section through an ovary of an adult *N. wheeleri*, collected in March, showing early stages of folliculogenesis (left) with thinner granulosa layer (g), central nucleus and small nucleolus (arrow) possibly prior to first meiotic division. Scale bar = 0.1 mm.

The oviduct enlarges at sexual maturity but is largest in gravid or recently gravid specimens when it becomes both broad and long and may be gathered in folds. The secretory portions develop in synchrony with gravidity (Figure 4.24). Although various 'crevices' and evaginations (possible sperm retention sites) were seen in the wall of the oviducts (Figure 4.24) no sperm were seen in any of them.

4.3.3 Development of Gonads and Seasonality of Reproduction

Only representative data and charts are presented for *N. levis* and *N. laevissimus*. Data comparing specimen collection dates with SVL in *N. levis* (Figure 4.25) show that adult specimens have been collected throughout the year except in mid-winter. Hatchlings are present in late summer and autumn and young juveniles in most months of the year.

Testis volume in *N. levis* (Figure 4.26) shows an abrupt increase at about 50 mm SVL (sexual maturity). Both mean and maximum testis size (for SVLs greater than 50 mm) continue to increase with SVL. Enlarged testes occur in spring, summer and autumn in both *N. levis* and *N. laevissimus* (Figure 4.27). The pattern of testis growth in *N. laevissimus* (Figure 4.28) shows sexual maturity is reached at 49 mm SVL and that the testes of larger adults increase in size with increasing SVL. The testes in *N. laevissimus* never return to the small size found occurring in *N. levis*. The testis sizes are similar for the left and right testes of *N. levis* (Figure 4.29), with a high correlation of $R^2 = 0.876$.

Enlarged ovarian follicles (>2.8 mm diameter) in *N. levis* first appear at 74 mm SVL (Figure 4.30). Data show that enlarged follicles may occur in all seasons of the year, but not in mid-winter, in *N. laevissimus* (Figure 4.31).

Onset of sexual maturity occurs from 54 mm SVL in male *U. milii* (Figure 4.32), but the minimum adult testis size (in a sample) remains small even in large adults. *U. milii* (Figure 4.33) shows that small or enlarged testes can occur in all seasons of the year. One *U. milii* showed enlarged ovarian follicles at 61 mm SVL (Figure 4.34) (this specimen was collected in November), however enlarged follicles were not found in other specimens until they had reached 72 mm SVL. There is a large

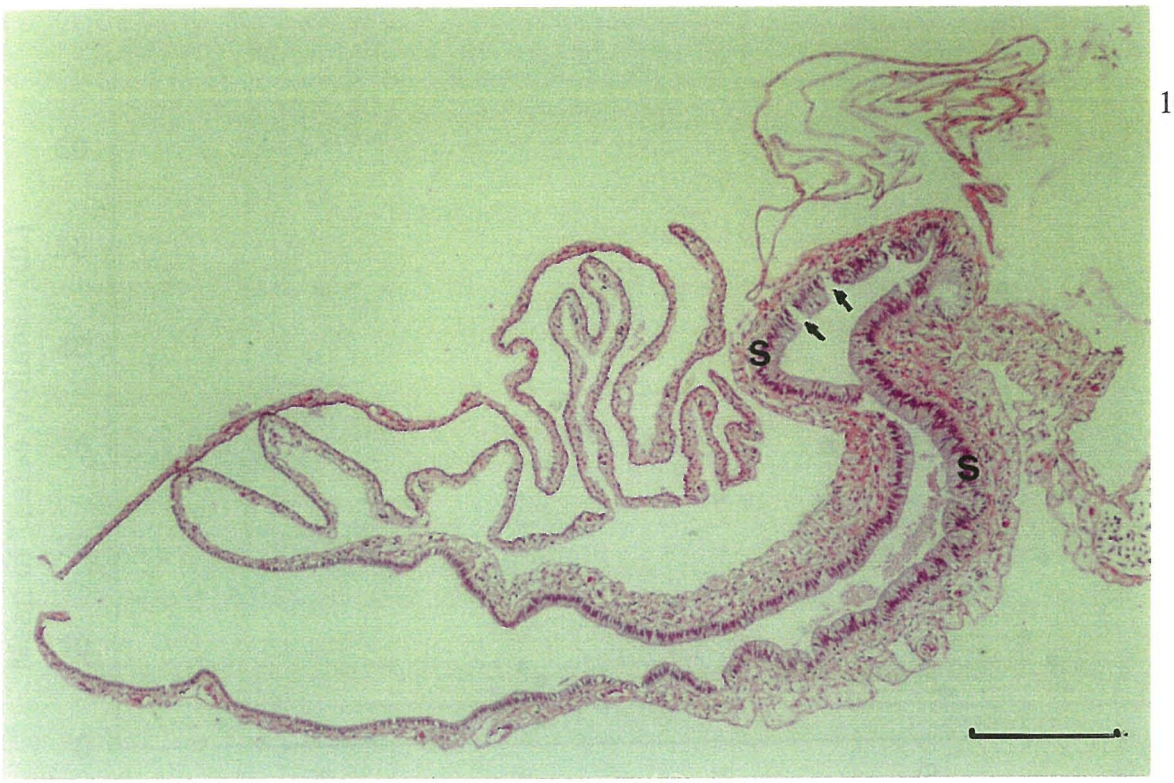

Figure 4.24 Section through oviduct of an adult *U. milii* R69845, collected in January, showing progestin induced hypertrophy and thickened secretory epithelium of shell gland (S). The small cavities (arrows) may be seminal receptacles, but no sperm was seen in them. Scale bar = 0.5 mm.

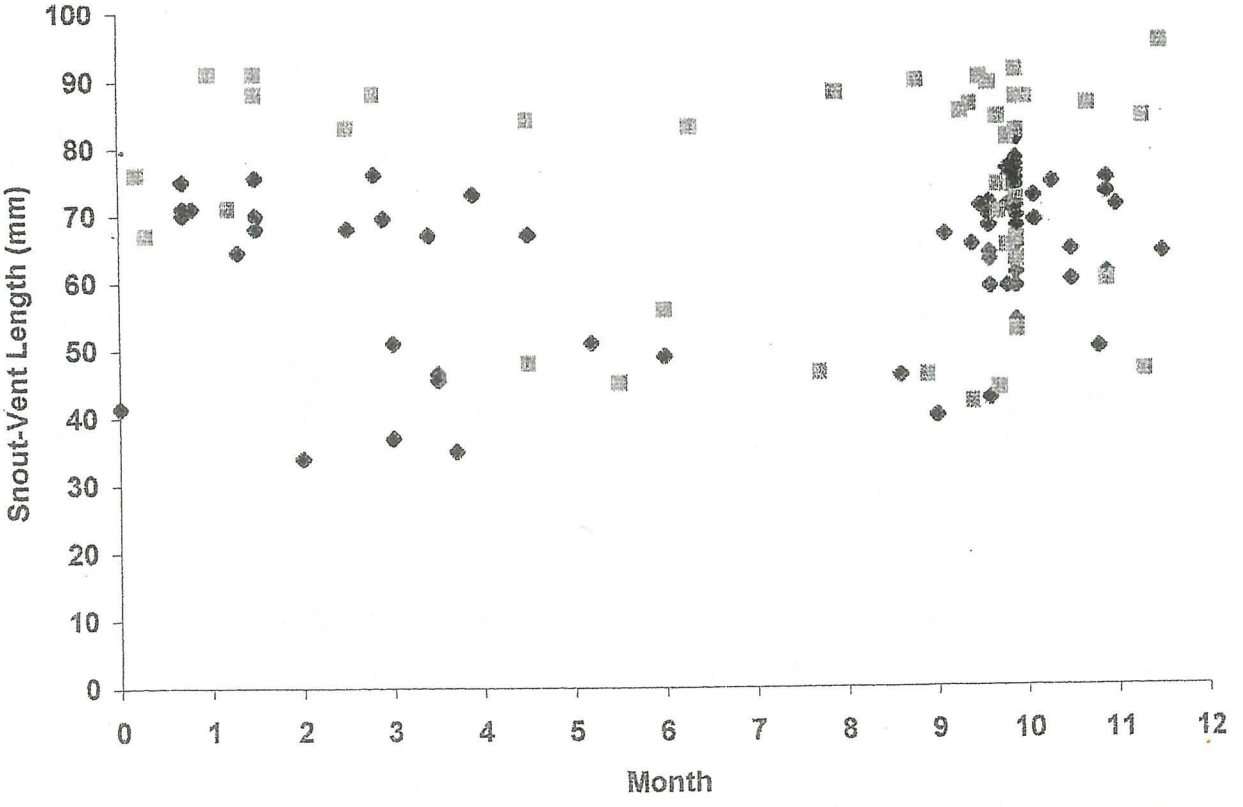

Figure 4.25 Collection dates and SVL in *N. levis* showing a brief mid-winter gap in collection of both males (diamonds, N = 67) and females (squares, N = 44).

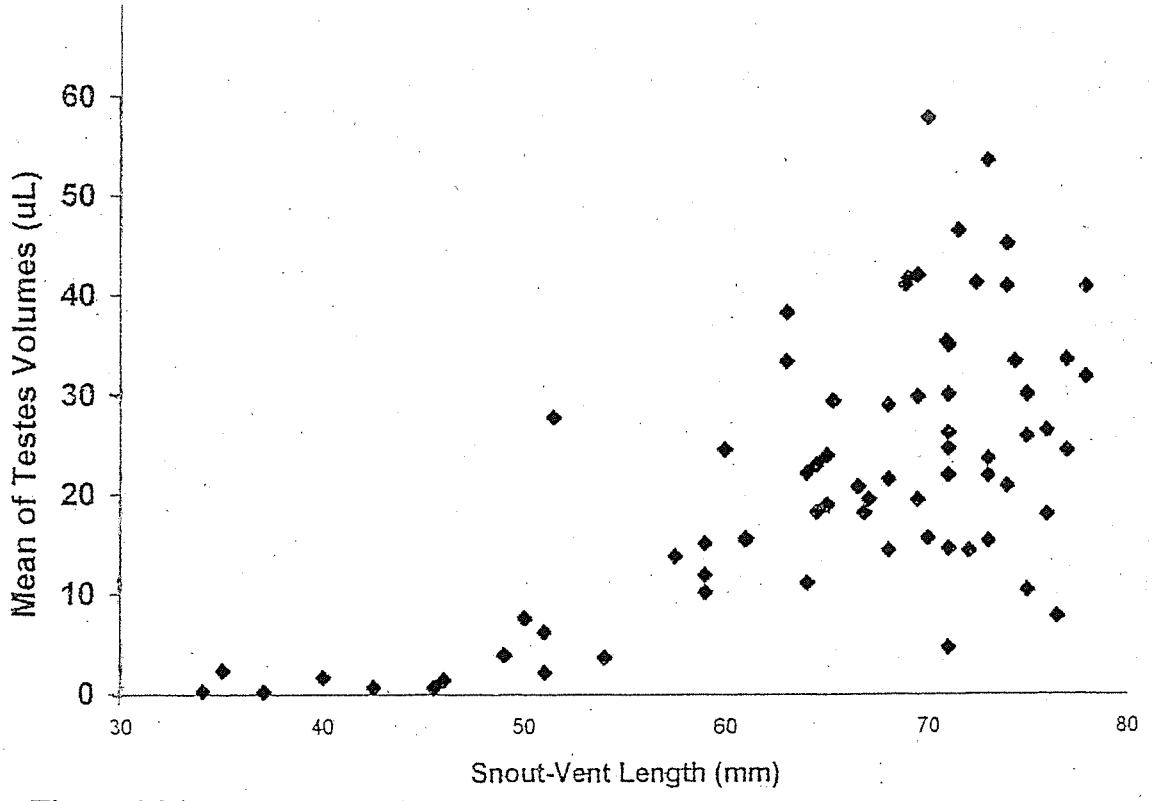

Figure 4.26 Mean volume of both testes and SVL in *N. levis* showing onset of sexual maturity at approximately 50 mm SVL and increasing testis size range with increasing SVL (N = 73).

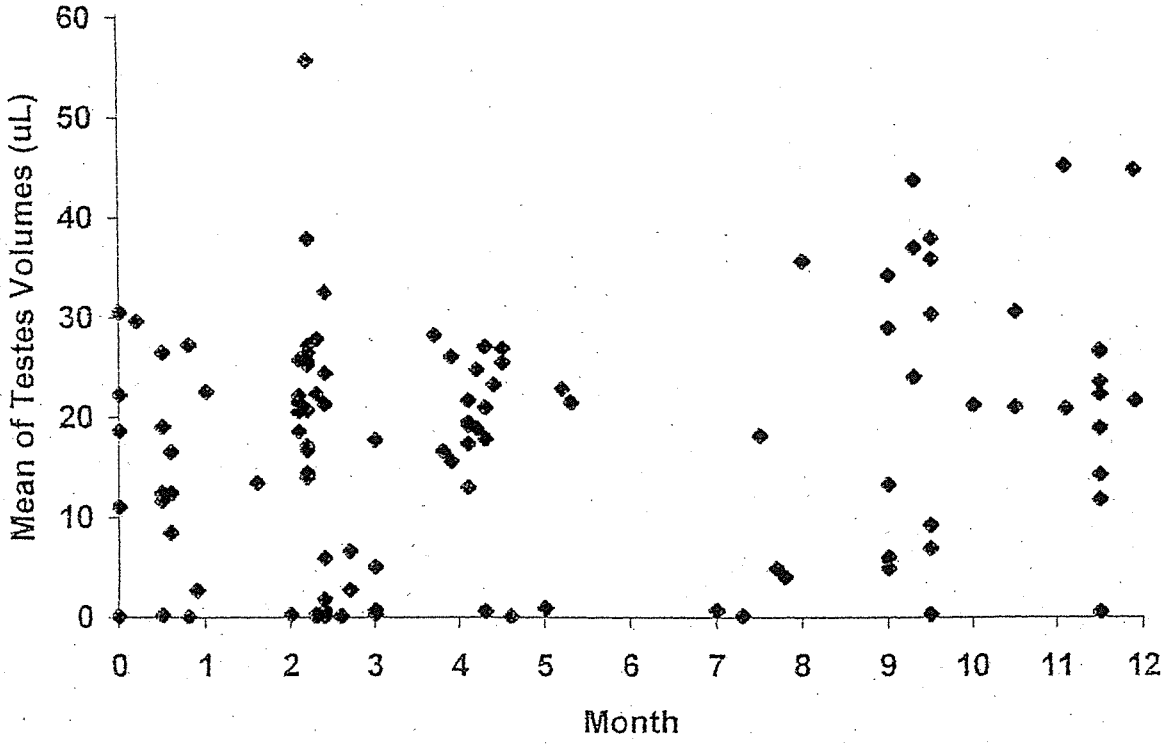

Figure 4.27 Collection dates and testes volumes for *N. laevissimus* showing enlarged testes over most of the year and only a short mid-winter gap in collections (juveniles included) (N = 114).

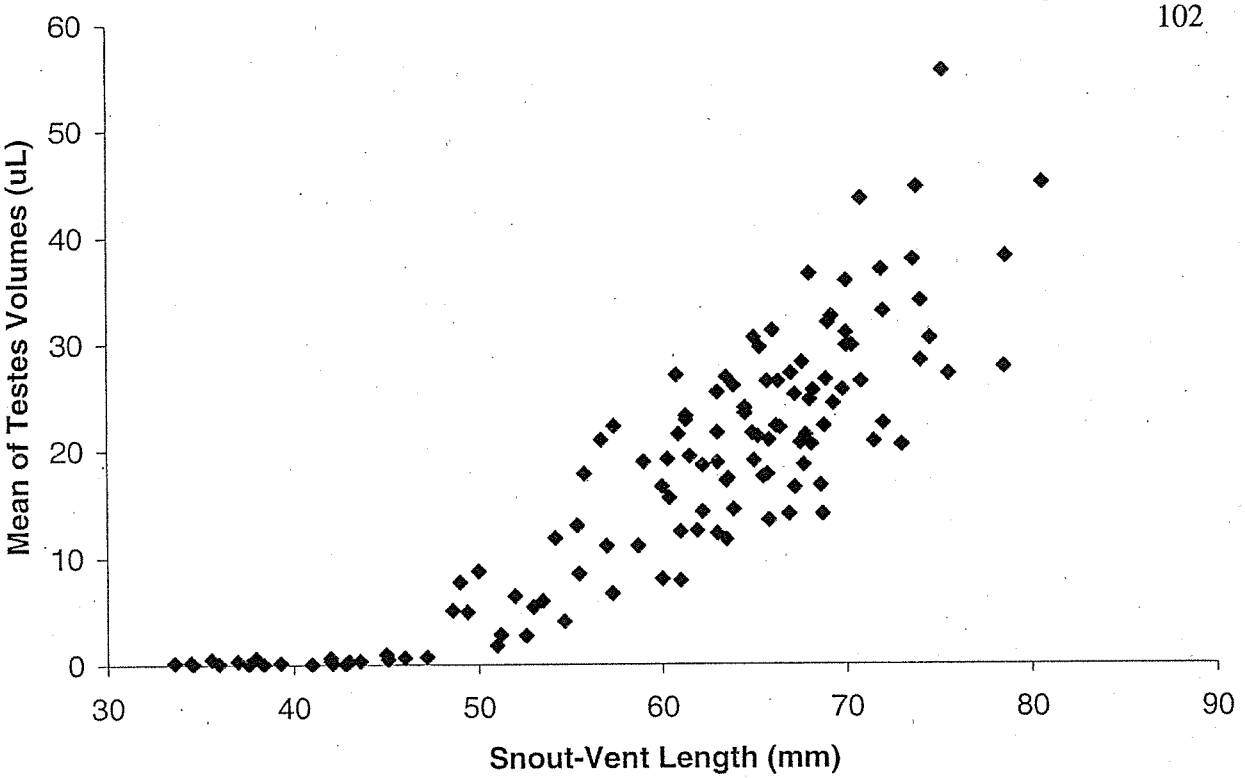

Figure 4.28 Mean testis volume and SVL in *N. laevissimus* showing onset of sexual maturity at 49 mm. Larger adults show no evidence of a quiescent period (N = 123).

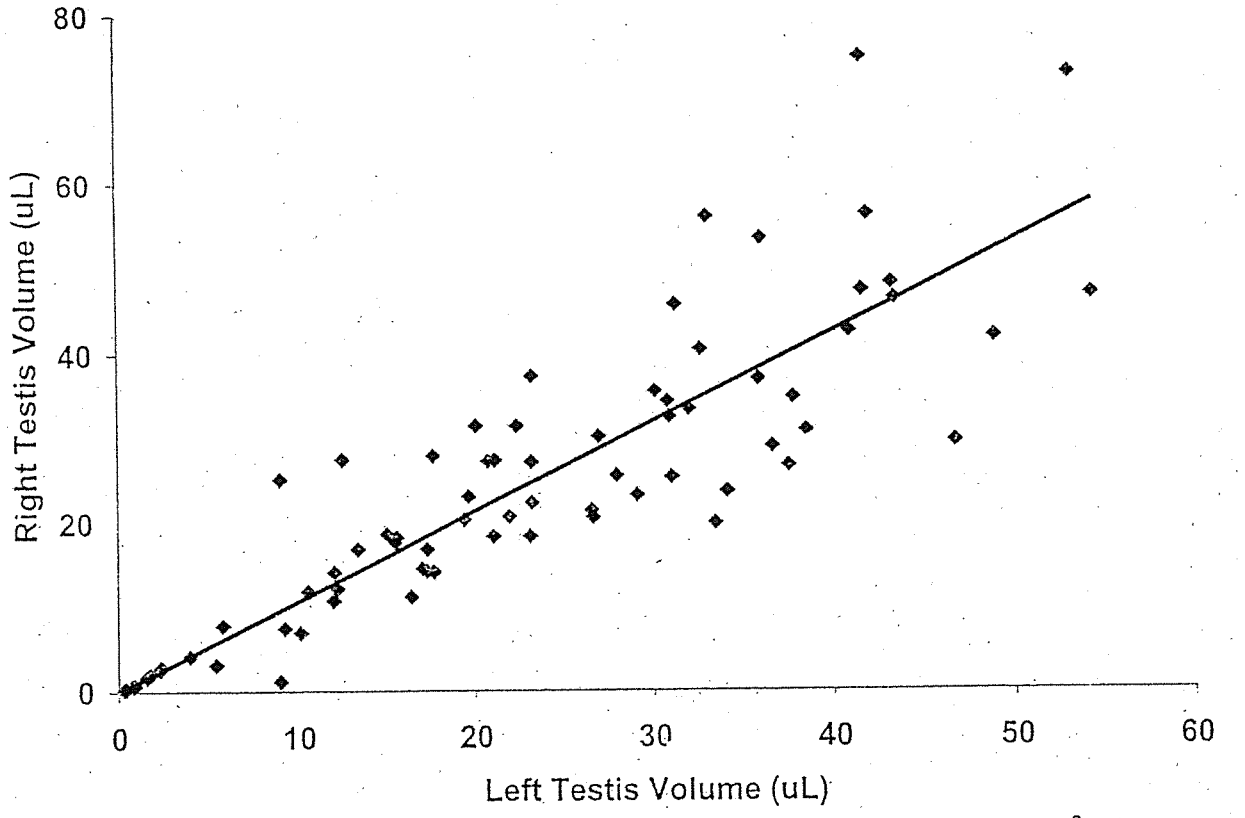

Figure 4.29 Comparison of volumes of left and right testes in *N. levis* (N = 73, $R^2 = 0.876$) and the linear regression line is given by y = 0.040x + 0.856).

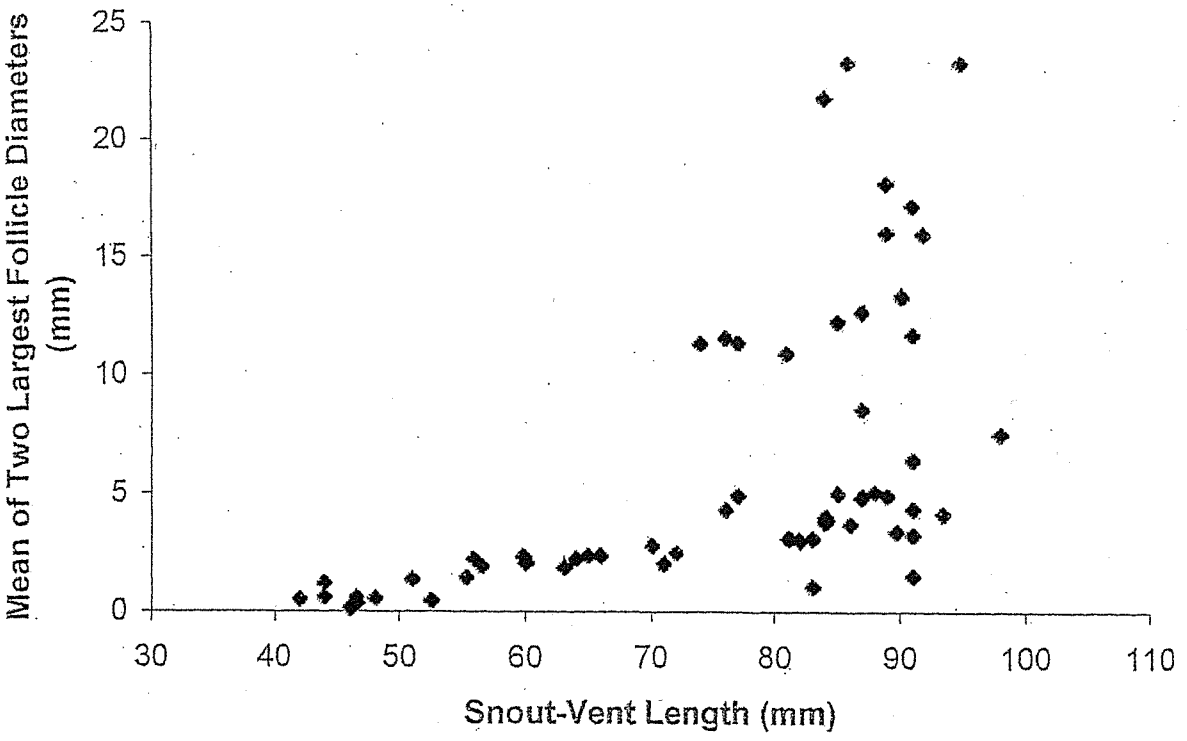

Figure 4.30 Ovarian follicles and SVL in *N. levis* showing that sexual maturity is reached at 74 mm SVL (N = 53).

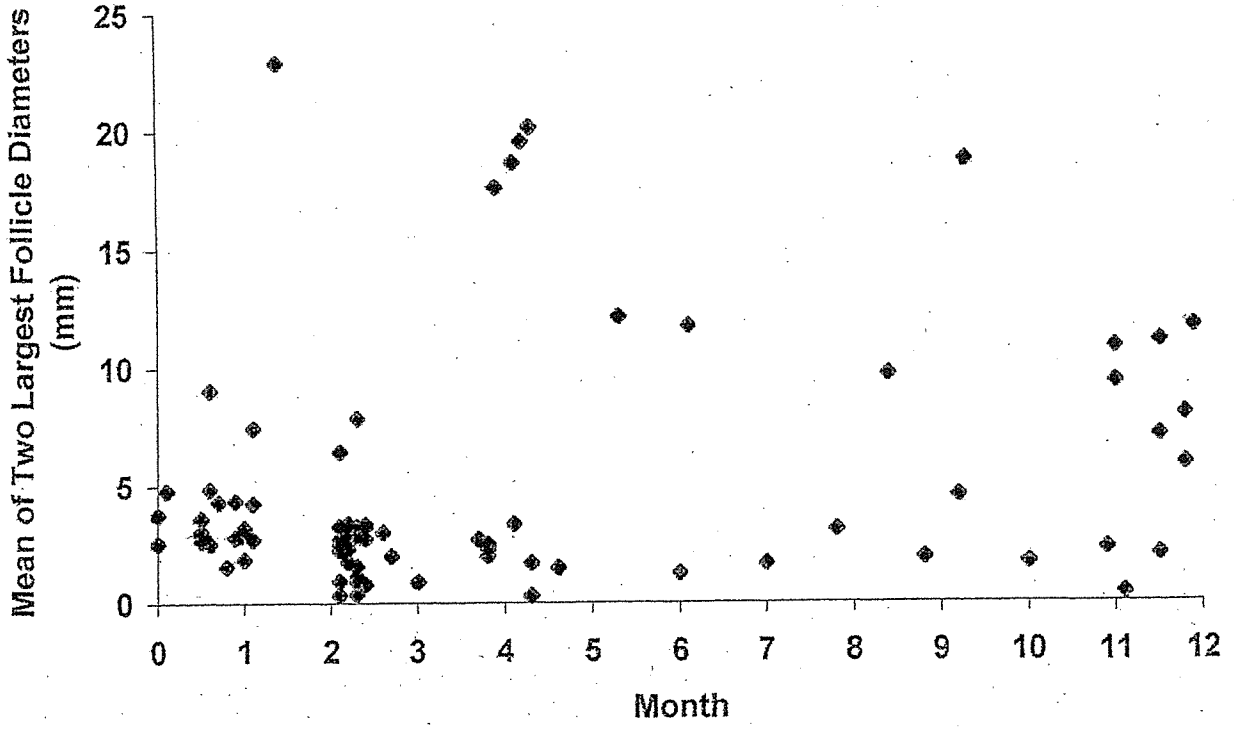

Figure 4.31 Collection dates and follicle size in *N. laevissimus* showing enlarged follicles or gravid females in every month of the year except July (N = 88).

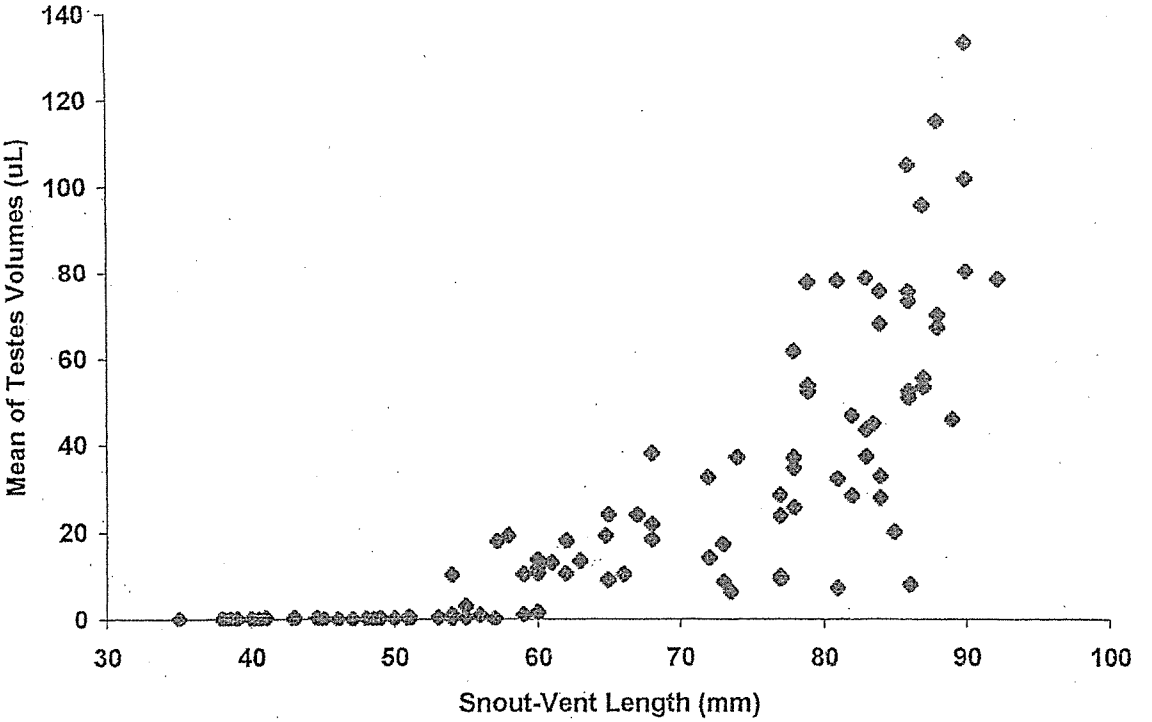

Figure 4.32 Testis size and SVL in *U. milii* showing onset of sexual maturity at 54 mm SVL. Some adult testes may be very small (N = 97).

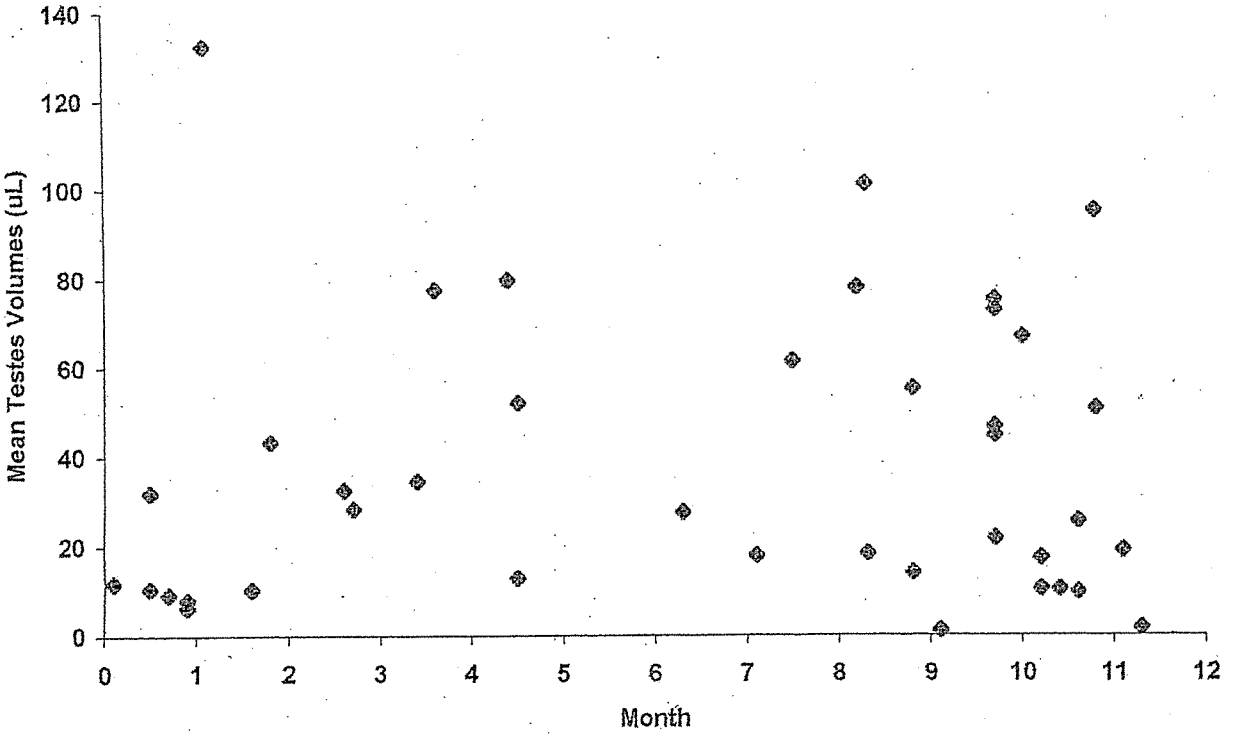

Figure 4.33 Collection dates and testis size in *U. milii* adult males (60-92mm SVL) showing enlarged and small testes in all seasons (N = 40).

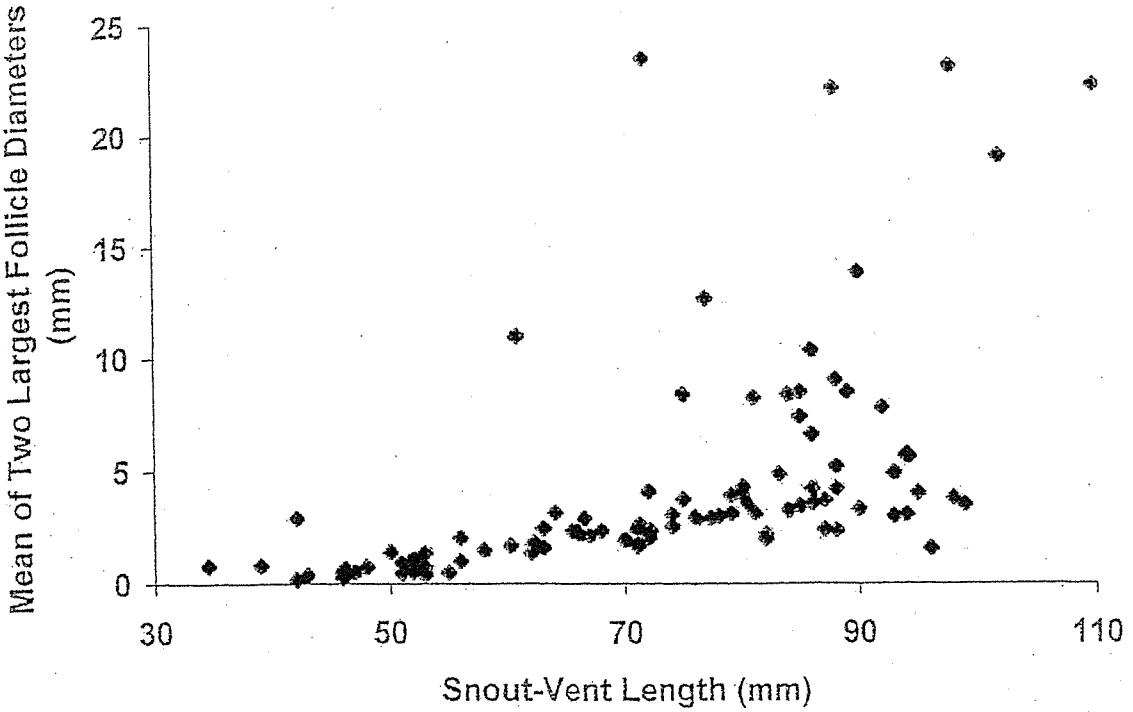

Figure 4.34 Ovarian follicle size and SVL in *U. milii* showing that sexual maturity is reached at 61 mm SVL (this may be exceptional). The largest specimen was gravid (N = 107).

variation in adult ovarian follicle size. The largest left and right side ovarian follicle diameters in *U. milii* (Figure 4.35) show a high correlation ($R^2 = 0.918$). Comparison of collection dates and Ovarian follicles in *U. milii* (Figure 4.36) are enlarged mainly between mid-winter and mid-summer. A summary of the maximum SVL values and minimum sizes (or estimated sizes) at sexual maturity for males and females *of Nephrurus* and *Underwoodisaurus* is given in Table 4.1.

All the eggs laid under seasonal conditions in captivity (*N. amyae, N. asper, N. deleani, N. levis, U. milii, U. sphyrurus*) and the vast majority of gravid museum *Nephrurus* and *Underwoodisaurus* specimens showed that egg-laying occurs largely in spring and summer.

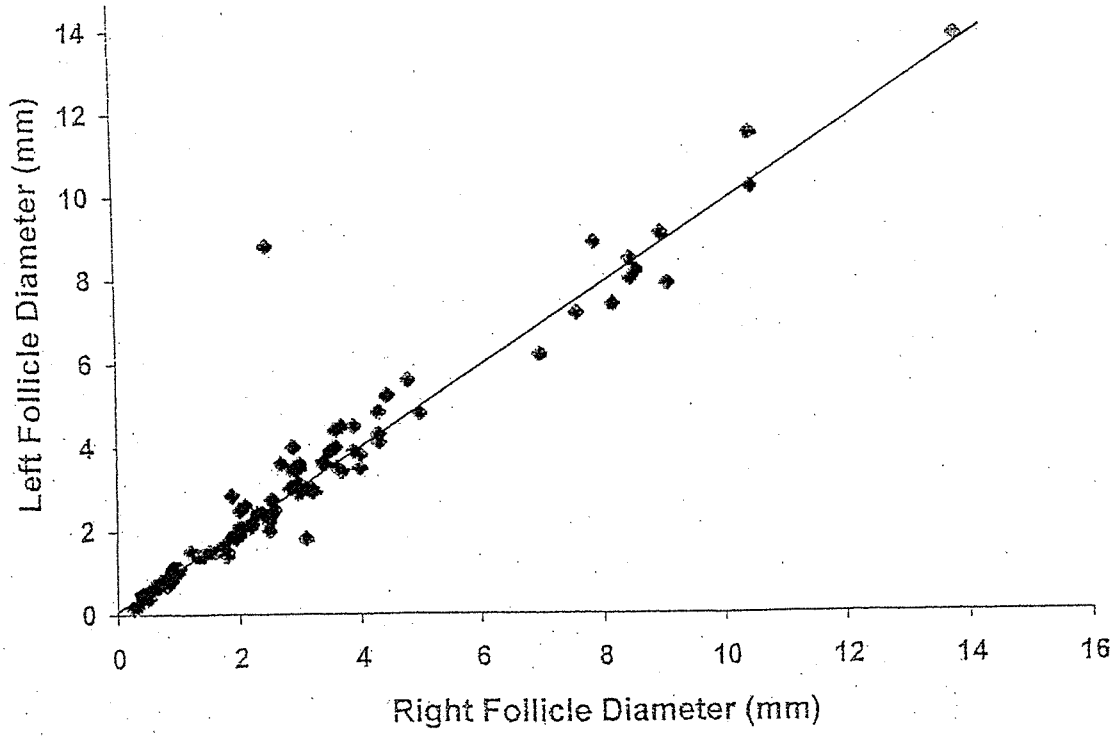

Figure 4.35 Comparison of left and right follicle sizes in *U. milii*, $R^2 = 0.918$, and the linear regression line is given by $y = 0.975x + 0.207$, (N = 90).

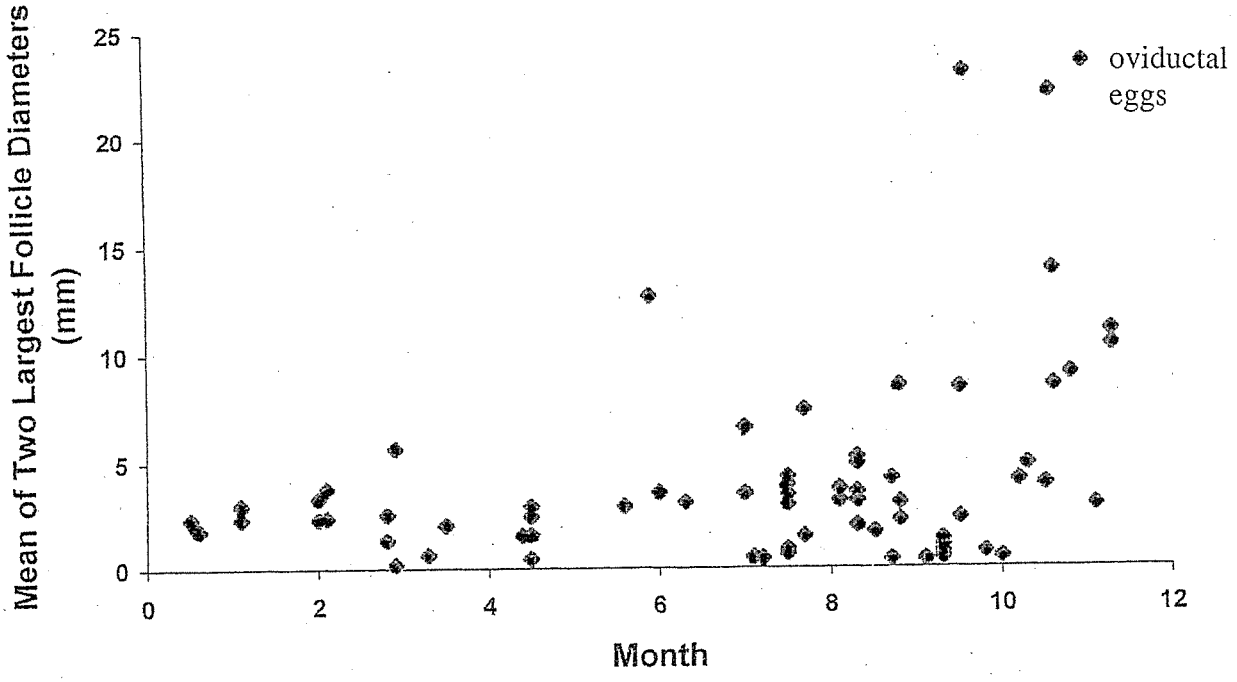

Figure 4.36 Collection dates and follicle sizes in *U. milii* showing that enlarged follicles (>4 mm diameter) occur mainly between mid-winter and mid-summer (N = 73).

Table 4.1 Size at sexual maturity in *Nephrurus* and *Underwoodisaurus* geckos. Values in parentheses are percentages of maximum SVL for the gender.

SPECIES	Maximum SVL Females (mm)	SVL Size of Females at Maturity (mm)	Maximum SVL Males (mm)	SVL Size of Males at Maturity (mm)	N (Adults) Females/Males
N. amyae	137	97 (71)*	110	69 (63)	6/8
*N. asper****	118	76 (65)	104	63 (61)	22/32
N. deleani	89	63 (71)*	67	42 (62)*	9/7
N. laevissimus	95	66 (69)	81	49 (61)	75/110
N. levis	98	74 (75)	81	50 (62)	48/78
N. sheai	121	86 (71)*	100	63 (63)	14/10
N. stellatus	86	61 (71)*	78	48 (62)*	5/19
N. vertebralis	90	64 (71)*	78	45 (58)	9/31
N. wheeleri	103	78 (76)	83	51 (62)*	13/16
Mean *Nephrurus* SVL as % of maximum		**(71.3)**		**(61.3)**	
U. milii	110	72 (65)**	95	57 (60)	95/108
U. sphyrurus	85	56 (67)	73	44 (60)*	10/12

* Estimates based on mean value for the genus (*Nephrurus* or *Underwoodisaurus*).
** One apparently precocious female *U. milii* was found with enlarged ovarian follicles at 61 mm SVL, or 55% of maximum (not used in calculations).

4.4 Discussion

4.4.1 Courtship and Mating Behaviour

Mating in *Nephrurus* and *Underwoodisaurus* species occurs in a typical primitive saurian fashion (Böhme and Bischoff, 1976, Wilson and Knowles, 1988). This primitive behaviour is compatible with other plesiomorphic gekkonoid characteristics such as nocturnality, vertical pupil, usually procoelous vertebrae, numerous small pleurodont teeth, facial artery passing through or anterior to stapes and a number of other skeletal features (Underwood 1957; Estes, 1983; Estes and Pregill, 1988). The mating behaviour of *Nephrurus* and

Underwoodisaurus species was similar, possibly more prolonged and complex in *Nephrurus* species, but more observations are required to define 'normal' mating behaviours. The arched tail position adopted by female geckos on the approach of a conspecific adult male gecko was observed on four occasions and may indicate a sexually receptive signal to the male.

The observation of a male *N. asper* briefly mistaking a female *N. levis* for a female *N. asper*, then recognising the mistake at a distance, (without evidence of auditory or pheromonal cues) suggests that visual cues are important in mate recognition. The observations of sniffing and licking also indicates a possible component of olfaction and gustation, which may trigger further courting and mating reflexes. Touching of the female by the snout of the male was always a component of courting and mating behaviour, suggesting that epidermal mechanoreceptors may also be significant in determination of courting and mating behaviour. The licking behaviour by courting males (and females) suggests that conspecific and perhaps sex recognition is at least partly by taste. Visual, pheromonal and perhaps other cues may also be important in combination.

The dramatic manner in which a courting male *N. asper* was instantaneously stopped when the female brushed her caudal knob around his snout suggests a possible pheromonal mating inhibition function of the knob. The caudal knob of *N. stellatus* is well supplied with subcutaneous capillaries (Wayne, 1996). Wayne (1996) discounted the pheromonal function of the caudal knob on the basis of inadequate vascularity, although his SEM photograph shows a dense capillary bed in the caudal knob. A dense capillary bed in the caudal knob also occurs in *N. levis* (small knob) and *N. asper* (large knob) (Russell and Bauer, 1987b). Hence, in the absence of other active tissue such as muscle (Russell and Bauer, 1987b), a pheromonal function for the knob is a strong possibility.

No observations were made that could directly suggest any function of the preano-inguinofemoral tubercles found in adult males of the derived *Nephrurus* group. However, the structure and position of the tubercles only in adult males suggests that they probably function in relation to reproduction. Possible functions include 1) territory marking (Miller, 1984; Allen, 1987), 2) mate recognition, enabling females to recognise appropriate adult males, 3) ovulation triggering in females, 4) mating receptivity trigger, where females are

triggered to adopt mating receptive behaviour. Territory marking is unlikely in view of the shifting sand habitat, and also the absence of territorial behaviour of these species. Mate recognition is possible but the fact that males find females that do not have the tubercles suggests that this function may not be the answer. Ovulation triggering is also thought unlikely because ova could be wasted if mating did not take place. Triggering of mating receptivity in the female is a likely function on the evidence. If there is a pheromonal mating inhibitor, then a mating trigger is also a logical possibility. The functions of the tubercles would be reduced in cold weather and it would facilitate appropriate matings with fertile conspecifics in a dispersed population of low density.

How only one hemipenis on the correct side is everted is not known, but the way the male approaches the female from one side may stimulate eversion of the correct hemipenis. Ipsilateral stimulation of the cloacal spurs by contact with the female may trigger hemipenial eversion (Rieppel, 1976), presumably under autonomic control and involving a muscular/ haemodynamic control mechanism (Kent, 1975). Well developed cloacal spurs are present only in adult male geckos, suggesting association with mating, possibly to provide appropriate tactile stimulation of the female or to hold open the female cloaca (Henkel and Schmidt, 1995), as well as triggering the hemipenis to erect. It is unlikely that the cloacal spurs are needed to hold open the female cloaca because some geckos (the Sphaerodactylinae) like many other lizards, lack cloacal spurs (Rieppel, 1976; Patchell and Shine, 1986). The fact that eversion of the hemipenis does not occur except during mating, suggests that the reflex is complex and may involve cortical as well as spinal or local components. The cloacal spurs are well developed in the males of *Nephrurus* and *Underwoodisaurus*, with marked interspecific variation in morphology (Bauer, 1986). Nevertheless, my observations of mating behaviour do not clearly show a specific mating related function for the cloacal spurs.

The bite by male *Nephrurus* and *Underwoodisaurus* species on the nape or shoulder region of the female during mating (as occurs in many lizard species) is considered to be the primitive mating grip position (Böhme and Bischoff, 1976). There is little significance in this grip because it is so widespread among lizards, although it may indicate that associated reproductive behaviours are also primitive.

The post-mating behaviour by the male gecko of licking and tugging at the hemipenis will help to ensure that substrate material that would cause irritation and possibly infection is not retracted into the hemipenial sheath. This behaviour may also assist in the correct repositioning of the retracted hemipenis.

The immediate post-mating receptive behaviour (presumed) of females indicates that females are prepared to mate more than once with different males. Because of the restricted clutch size, repeated matings would be of limited use in increasing gene diversity, but it may serve to increase the amount of stored sperm for future clutches, and provide sperm competition or negate the effect of male sterility (Olsson and Shine, 1997). This character may be a selective advantage to solitary species or where the population density is low, especially in cases where the onset and duration of the seasonal reproductive window is variable.

The cold soil surface temperatures of central and southern Australia probably preclude reproductive activity of lizards in winter (James and Shine, 1985). The fact that *U. milii* laid eggs in late autumn and winter when kept warm (with a 12 hour light cycle) suggests that temperature is a significant trigger for reproduction. The over-winter retention of eggs or sperm (at least in *Nephrurus* species) (Harvey, 1983; Couper, 1996) suggests that earlier oviposition in spring may occur. Juveniles that are able to grow larger and build up a larger lipid energy reserve prior to the onset of their first winter are more likely to survive (Daniels, 1984). There is no indication that prolonged egg retention is a common occurrence, although a degree of egg retention may occur while the female is searching for a suitable nesting site (Andrews and Sexton, 1981) or if conditions are inappropriate for egg laying. The finding of female *N. laevissimus* with enlarged yolked follicles in autumn suggests possible late season mating and over-winter egg retention or sperm retention in this species. However the data (for all species) do not allow a clear conclusion on whether late season mating or over-winter sperm storage occurs. The fact that female *U. milii*, isolated after laying eggs, do not lay more eggs, despite apparently favourable conditions, gives no support for the suggestion that *U. milii* may have sperm retention (How et al., 1990). It is possible that sperm retention only occurs sporadically or under certain restricted conditions.

Another possibility is that mating triggers ovulation in *U. milii* (in which case sperm storage is not an issue). No ovulated but totally unshelled eggs were found in this study, suggesting that the interval between ovulation and shelling is very short. If egg-laying in the wild is iteroparous it would be expected that either the females would mate a number of times (as in many anoles, Tokarz, 1988) or that sperm retention might be required (as in at least some *Nephrurus* species (How et al., 1990; Couper, 1996)) to ensure fertilisation. Further study is required to determine whether sperm storage is used in *U. milii*.

4.4.2 Reproductive Cycles

Reproductive activity in most temperate oviparous lizards is restricted to the warmer spring and summer seasons (Fitch, 1970; Heatwole and Taylor, 1987), although some temperate lizards also have reproductive activity in autumn/winter (Guillette and Mendez de la Cruz, 1993; Heatwole and Taylor, 1987). *Nephrurus* geckos are reproductively active throughout much of the year in the north of Australia (How et al., 1990). The follicle and testis size evidence in this study for *N. laevissimus* is that both males and females may be reproductively active throughout most of the year, probably excluding only July and August. The finding that adult female *Underwoodisaurus* have enlarged follicles through much of the second half of the year whereas adult males have enlarged testes throughout most of the year (except march, June and July) suggests that sperm storage may occur in this species but that egg-laying occurs mainly in the spring and early summer. More data are, however, needed for all *Nephrurus* and *Underwoodisaurus* species especially for the winter months to precisely define their reproductive cycles and determine how they vary geographically.

If very large sample sizes were available it might be more appropriate to take the mean size and standard deviation of all specimens between the minimum with, and the maximum without, mature gonadal development when making interspecific comparisons of size at sexual maturity. In this study the sample size was limited in most species and therefore the minimum SVL, for a given species, at which enlarged gonads could be demonstrated was chosen as a more appropriate definition.

Among female geckos the problem of identifying minimum size at sexual maturity is confounded because, in addition to natural variations in size at sexual maturity, the ovary is

likely to be quiescent for a major portion of the year, hence, even larger sample sizes of adult females are needed to accurately determine minimum size at sexual maturity.

The seasonal changes in absolute and relative testicular volumes among adults of all *Nephrurus* and *Underwoodisaurus* species are likely to represent variations in reproductive activity (Sanyal and Prasad, 1965; Licht, 1971; Chiu and Maderson, 1975; Dell and Chapman, 1981). These variations are induced by hormonal fluctuations between breeding and non-breeding periods or seasons (MacAvoy, 1976; van Wyk and Mouton, 1996), thus suggesting large within-season fluctuations in secretion of anterior pituitary gonadotrophin. The frequent occurrence of reproductively inactive adult males and females of all the study species during the breeding seasons suggests the possibility of a long population breeding season, but with a possibly short individual breeding season. This may be an important reproductive strategy in *Nephrurus* and *Underwoodisaurus* in sustaining populations, particularly in areas and times of adverse and variable meteorological, edaphic and ecological conditions, by increasing the probability of hatchling survival each season.

The differences in sperm numbers between the seminiferous tubules and the epididymis found in *Nephrurus* and *Underwoodisaurus* could result from either recent copulation or very recent onset or cessation of testicular activity. Differences in sperm number category states between specimens in similar seasons suggest that there may be some delay in the passage of semen both into and out of the epididymis, and also that factors other than time of year, such as temperature or nutritional status, may be involved. The epididymal acidophilic colloid must be secreted by the epididymis, as it is not found in the vasa efferentia; the cells of the wall of the epididymis are secretory in appearance (cuboidal to columnar with granular cytoplasm), whereas those of the vasa efferentia are not (mainly cuboidal or simple squamous with less granular cytoplasm). The colloid probably breaks down by enzyme action (Guyton and Hall, 1996). The presence of areas of breakdown, or lacunae, both at the periphery of the colloid and from within the colloid (adjacent to sperm cells) suggests that the origins of this enzyme may be from the wall of the duct as well as from the sperm cells.

The close correlation of the volumes of contralateral testes *e.g.* in *N. levis* indicates that the changes in volume are synchronised. The synchronisation is presumably mediated by the

presence of similar densities of gonadotrophin and androgen receptors in the testes (Norris, and Jones, 1987). Large asymmetry of testis size in a few specimens probably represents normal morphological and perhaps physiological variation, although some differential shrinkage and/or compression during preservation cannot be ruled out. The largest specimens of most *Nephrurus* and *Underwoodisaurus* species had less than the average adult testicular volume, but none of them had a quiescence level testis volume, so there is no firm evidence of testis senescence with age. The slow growth observed in several older geckos suggests that growth continues in an asymptotic manner. If reproductive senescence occurs, it would be found in the largest specimens, although natural variation in SVL will confound the relationship unless senescence is pronounced. The finding of minimal evidence for reproductive senescence (*i.e.* atrophy of gonads in largest specimens of each species) suggests that the reproductive life is prolonged (possibly over a period of about 10 years, personal observation) in both males and females of *N. levis* and *U. milii*.

Reptilian endocrine control mechanisms are poorly understood (Ferguson, 1985; Zug et al., 2001), but fundamental processes are presumed to apply. The pituitary controls the large difference in maximum size of follicles found in breeding and non-breeding individuals (Jones and Baxter, 1991) and the rapid deposition of large amounts of yolk just prior to ovulation (Noble, 1991). The sequence of follicle sizes found in an ovary probably indicates that the endocrine influences of the anterior pituitary follicle stimulating hormone and luteinizing hormone on the ovary (Norris and Jones, 1987) have trophic effects on all developing follicles even though only the largest is released at ovulation. The trigger mechanism for initiating vitellogenesis is uncertain but probably involves several factors such as plasma oestrogen levels, temperature and possibly day length.

The size sequence of ovarian follicles suggests that the second largest pair is already at an early to middle stage of vitellogenesis prior to ovulation of the largest pair, if conditions are suitable. Thus, the interclutch interval must involve both the final stages of development of vitellogenesis prior to ovulation, as well as oviductal egg formation.

Unlike the avian ovary, the germinal bed of saurian ovaries contains mitotically dividing oogonia, oocytes and primordial follicles (Guraya, 1989). Few oogonia were observed in ovarian sections, indicating few mitotic divisions prior to folliculogenesis. Oogonia in

many reptiles begin entering meiotic prophase I in embryonic ovaries and continuing into adulthood (Boyd, 1940; Guraya, 1989). The occurrence of the first polar bodies in large, nearly mature follicles of *N. levis* shows that meiosis is suspended during vitellogenesis, and that completion of meiosis (ovum maturation) occurs rapidly (Guraya, 1989). At this stage, the nucleolus is larger than in the early stages of folliculogenesis (compare Figures 4.22, 4.23), indicating increased RNA synthesis. The nucleolus contains various granular elements, possibly as a prelude to germinal vesicle breakdown, indicated by the presence of a clear peri-nuclear zone (Guraya, 1989). The first polar body shows very little chromatin or karyoplasm. The actively growing follicles tend to be spherical and whitish when small, but become cream then yellow as they enlarge with the deposition of more yolk. In *Nephrurus* species, the number of growing follicles in an adult or sub-adult ovary at any time varies from 4 - 10, suggesting variation in reproductive status in time. The range of follicle sizes also suggests that the early stages of development are slow compared to the final stages, where large amounts of yolk material are transferred in a few days or possibly weeks.

The corpus luteum influences oviposition in squamates by acting on the Mullerian ducts and/or on the brain (Jones and Baxter, 1991), presumably via the hypothalamus, which controls gonadotrophin secretion from the adenohypophysis (Bellairs and Bryant, 1985; Yaron, 1985). All oviparous reptiles studied have steroidogenic corpora lutea in which progesterone is a major component of luteal secretion (Jones and Baxter, 1991). In many oviparous lizard species, including geckos, it is usual for the corpora lutea to regress prior to oviposition *e.g.*, *Lepidodactylus lugubris* (Jones et al., 1978), *Hemidactylus frenatus* (Jones and Summers, 1985). The normally short gravidity period of *Nephrurus* may be one reason that few corpora lutea were seen in this study. The small size and lack of distinctiveness in the corpus luteum may indicate that the requirements for progesterone are limited, possibly because of the brief period between fertilisation and egg laying.

4.4.2.1 Reproduction in *Nephrurus* Species

Nephrurus levis have been collected throughout the year except for a short period in mid-winter (Figure 4.25). Hatchlings and young juveniles have been collected in spring, summer and autumn, thus supporting the hypothesis of a prolonged breeding season and

possible iteroparity. Based on collection dates, the central and southern species appear to have a dormant period of at least a month in mid- winter, although collection data are not adequate to confirm this in most species.

The minimum size at which sexual maturity is reached in male *N. levis* is approximately 50 mm SVL (Figure 4.26), which is 62% of maximum SVL for males. This compares with a mean value of 63.0 % for the six *Nephrurus* species for which adequate data are available (Table 4.1), and a published value of 62 mm SVL (73% of maximum) (How et al., 1990). Juvenile *N. asper* (males) in captivity reach sexual maturity by the end of the second season under optimum conditions (Wagner and Lazik, 1996). Live specimens show well-developed hemipenes, cloacal spurs and preano-inguinofemoral tubercles at 60 mm SVL. Because many *Nephrurus* and *Underwoodisaurus* hatchlings emerge in late summer and grow very little in winter, (particularly in cooler regions) they will probably not be able to grow to sexual maturity before the following autumn. Thus, in the wild, sexual maturity would probably not occur until the third season *i.e.* about two years of age. This has been confirmed in captivity for *N. asper* and *U. milii* kept in minimally heated vivaria (Appendix 3).

There is a large variance in size of testes in all adults (*e.g.* Figure 4.26), suggesting that there may be a temporal fluctuation in size even within the breeding season. The majority of values of adult testicular volumes in *N. levis* and *N. laevissimus* are skewed towards the lower end of the range, even though the majority of specimens was collected during the warmer months of the year (Figure 4.25), suggesting that the reproductive phases are of limited duration. A possible alternative explanation for the testis size pattern observed is that male reproduction is biennial or triennial. This is thought very unlikely because males in captivity mate several times a year, females in captivity reproduce several times a season and also such behaviour in small reptiles usually occurs at high latitudes (Rawlinson, 1975; Greer, 1989). Variation in testicular volume in *N. laevissimus* differs from that in *N. levis*, *U. milii* and other *Nephrurus* species for which reasonable data are available, in that there is less variation among larger adults. Also, there is no return to the small, presumed quiescent state, at any season of the year (Figures, 4.27, 4.28), suggesting that mating may occur almost year round. This matches the almost year round presence of enlarged follicles or gravid females. The reasons for the differences are not clear but may be related to the small

latitudinal range of *N. laevissimus* compared to *N. levis*, which could significantly alter reproductive strategies in northern and southern populations.

The volumes of left and right testes are well correlated in all species for which adequate data are available (*e.g.* Figure 4.29). Thus, sampling from only one side is appropriate in assessing testicular size and activity. There is no evidence of atrophic testicular senescence among the largest and presumably oldest *N. laevissimus*, although fertility is reduced (*i.e.* less or no eggs produced) in older *U. milii* geckos (personal observation).

Among females of *Nephrurus* species, sexual maturity is reached at a mean minimum size of about 71 % of maximum SVL, compared to about 61 % among males of *Nephrurus* species (Table 4.1). This pattern is consistent with a selective pressure for females to grow larger before starting reproducing so that they can produce larger eggs. The majority of the largest follicles in adult geckos at any one time are at or near to the resting quiescent diameters, indicating that enlargement of follicles is a transient phenomenon, even in the breeding season (Figure 4.30).

Collection date and SVL data suggest almost year round reproductive activity for *N. levis* and the presence of hatchlings and juveniles in spring, summer and autumn suggests breeding throughout the spring and summer (Figure 4.31). *Nephrurus levis* lays eggs in the wild in October, December and January (and probably November and February). In captivity, with minimal heating (Appendix 3), eggs are laid at any time from 22-August, through the summer until 6-March. Up to three clutches of eggs are laid each season in captivity, confirming the presence of seasonal iteroparity. In captivity, the reproductive life of *Nephrurus* geckos is at least five years and possibly (by comparison with *U. milii*, personal observation) as much as ten years.

4.4.2.2 Maturity and Reproductive Cycle in *Underwoodisaurus*

The following information is based largely on *U. milii* because of the large amount of material and data available for this species.

In comparison to *Nephrurus* species, *Underwoodisaurus* species females reach sexual maturity at about 66 % of maximum size, suggesting there is less selective pressure towards producing larger eggs. Male *U. milii* reach sexual maturity at a similar size relative to

maximum SVL (60 %), compared to six *Nephrurus* species (61 %) (Table 4.1, Appendix 4, Table A4.12). The hatchling testis increases in volume by a mean factor of over 600 times during ontogeny (similar in *Nephrurus* and *Underwoodisaurus*), showing the dramatic effects of testosterone. The fluctuations in the adult testis volume most likely represent fluctuations between breeding and non-breeding periods or seasons. Testis volumes are greatest in the spring and summer, but testes may remain large well into the autumn (*e.g.* Figure 4.33). The variation in volumes of juvenile testes may reflect normal variation in concentrations of gonadotrophic hormones, but also indicates that some specimens are reproductively more precocious than others. Interpopulational variation may contribute to this variation in testis size. The largest specimen had intermediate sized testes but the largest testes were found towards the upper limit of SVL. There is no evidence to support atrophic testicular senescence in the largest specimens.

Whether the single precocious female *U. milii* that reached sexual maturity at a minimum 55% of maximum SVL was abnormal is not known. Sexual maturity in unheated temperate climate vivaria occurs in the third or fourth season after hatching. Like all *Nephrurus* species, *U. milii* has a large variation in the dimensions of the ovarian follicles within adult females (Figure 4.34), indicating frequent alternation between breeding and non-breeding periods. These changes (in both males and females) suggest large and, in the adult, variable changes in endocrine control. The 11% difference in SVL at sexual maturity and at first reproduction (if not an abnormal event) may indicate that first ovulation of a young female occurs some time after initial increase in size of follicles.

The growth of follicles in the left and right ovaries is symmetrical and the mean diameters of the largest left side and right side ovarian follicles are not significantly different ($P = > 0.05$) (Figure 4.35), indicating symmetrical hormone receptor density in each ovary. This parallel development presumably indicates a close control of follicular development by the neuroendocrine system. Although *U. milii* (in captivity) reproduces several times in one season (personal observation), relatively few large follicles were seen in the adult museum specimens (Figure 4.34). Thus, the periods when fertilisation can occur may be short and the final follicle growth phase prior to shelling is probably rapid.

Adult females of *U. milii* may have at least moderately-sized follicles at any season of the year (Figure 4.36). Gravidity occurs from November - March, suggesting that *U. milii* has seasonal iteroparity in the wild (as confirmed in captivity). Oviductal eggs were excluded from the analysis because the linear regression would be skewed by the occasional finding of a female with only a single egg and also because eggs are far from spherical in shape. There is no overt evidence for any senescence effect in the ovarian follicles, such as a decrease in follicle size with the largest SVLs.

In *Underwoodisaurus* species (where there is no significant sexual size dimorphism) and in contrast to *Nephrurus* species, sexual maturity may be almost synchronous in males and females by the third or possibly fourth season.

4.4.3 Testes, Epididymides and Vasa Deferentia

The changes occurring in the reproductive system at sexual maturity are more obvious externally in the male, but less dramatic internally than in the female.

The large variance in the adult testis volume was found in all the study species, suggesting that this is probably normal anatomical and physiological variation. The finding of juvenile sized testes in some 'adult' male geckos, above the minimum SVL, suggests that some geckos experience a delay in the onset of sexual maturity relative to others. All of the testes of medium to largest adults had testes that remained above the juvenile size (in both relative and absolute terms) (*e.g.* Figures 5.26, 5.28, 5.32). Early sexual maturity in males may provide a selective advantage, particularly in sparsely distributed species, as it means that an extra, larger cohort of males is available for mating each season.

As is common with many endocrine tissues (Bennett and Mader, 1996), the testes of adult *Nephrurus* and *Underwoodisaurus* are highly vascular compared to other tissues. Although the major proportion of vascular tissue occurs in the superficial tunica vasculosa, small vessels penetrate the entire peritubular testicular structure (Figures 4.4, 4.6). This high vascularity presumably reflects the high metabolic rate of the seminiferous tubules during the breeding season and also the requirement to exchange hormones between the blood stream and the testicular tissues.

For *Nephrurus* species, where adequate data are available, the largest testes at any given SVL are approximately double the minimum volume at this same SVL, suggesting that this may be the normal breeding / non-breeding difference in testis size. In *U. milii*, the growth pattern of the testes is different from that of *Nephrurus* species, with an increase in relative testis size at sexual maturity followed by a period of slower growth until a SVL of about 80 mm is reached. After 80 mm SVL, there is a further large increase, possibly related to the third season after hatching (Figure 4.32). Although not confirmed in this study it is reasonable to assume that larger testes produce more sperm, one possible advantage of more sperm is that there is increased sperm competition for multiple-mating species. The minimum testis size (at a given SVL) in both small and large *U. milii* adults remains relatively low, but the maximum volume (at a given SVL) is greater in larger males (up to ~12 fold at 85 – 87 mm SVL). This compares with an annual testicular regression-recrudescence cycle of up to 10 fold in an unspecified Australian gecko species (King and Horner, 1993), up to 17.5 fold in *Hemidactylus bowringii* (Chiu and Maderson, 1975) and up to 300 fold in some birds (Storer, 1975). Testicular size (and presumably activity) in *N. levis* and *U. milii* varies from very low to high in all seasons of the year. Although the testes of southern species, such as *U. milii* and *N. levis*, are more consistently increased in size in the spring and summer, some enlarged testes were found in autumn and early winter (Figure 4. 33).

The presence of spermatozoa in the epididymides and vasa deferentia is often all-or-none (or almost none). Sperm numbers also vary along the various parts of the duct system. However, without more detailed knowledge of mating and sperm transport, it is difficult to draw conclusions relating to differences in sperm numbers or densities in the ducts, except that they may be 'active' (adult) or 'inactive' (adult or juvenile).

The various forms of the spermatogenous clones, such as 'feathering', 'tenting' and 'clumping', were not seen consistently in any one species and it is not known whether they represent sequential stages, anomalous variations or form part of the normal range of variations seen in spermatogenesis. The irregular variations occurring within a single specimen at one time suggest that the various stages of spermatogenesis do not occur as discrete rings or bands along the seminiferous tubules. The various arrangements of the spermatids in the seminiferous tubules may represent transient interspecific variations.

Another possibility is that they represent a sequential variation of the stages in maturation that occur in the junctional complexes attaching the spermatids to the underlying sustentacular cells (Fawcett, 1975).

The reduced numbers of seminiferous tubule sections seen across the middle of a juvenile testis compared to an adult testis suggest that, as well as an increase in testicular diameter, there is also an increase in length of the tubules by a factor of two or more with the attainment of sexual maturity. The importance of production of large numbers of sperm is suggested because 83.7 ± 9.3% of the preserved testis volume is occupied by convoluted seminiferous tubules. Whether the seminiferous tubules are branched, as in mammals (Williams and Wilkins, 1997), could not be determined, as no 'Y' shaped sections were seen. In testes of *Nephrurus* and *Underwoodisaurus*, there may be a number of seminiferous tubules that anastomose to form a single epididymis duct (as in some other lizards), but the degree of branching or anastomoses in a rete formation was not determined.

The vasa efferentia are very narrow, with very few sperm and very little secretory material present, indicating no sperm storage in this region and probably rapid transit. The thin wall indicates lack of secretory or absorptive cells or propulsive smooth muscle, but the ducts are lined with ciliated epithelium, indicating a possible mechanism of sperm transport (although these may be non-motile stereocilia, as found in mammals).

In mammals, the functions of the epididymides include sperm transport, maturation and storage, reabsorption of fluid, secretion of colloid, and probably absorption of old or defective sperm cells (Rhoades and Pflanzer, 1992; Guyton and Hall, 1996). The functions of the vasa deferentia are largely sperm transport, storage and some secretion (Kent, 1975). Their functions in reptiles are probably similar, and may include hormone secretion. They must also guide the sperm through several of the final stages of development.

How long the spermatozoa can remain viable while stored in the vasa deferentia is not known. The observations that large numbers of spermatozoa were found in all seasons of the year and that the sperm can remain viable in the female reproductive system for months (Couper, 1996) suggest that they may also remain viable in the male reproductive system for a period of months.

Variations in diameter of the adult epididymis are due partly to degree of distension by the contents, partly to variation of function along the duct, and partly to changes in cellular activity with reproductive activity. The thicker (pseudostratified) parts of the wall are probably associated with secretion and possibly dissolution of the colloid, as these cells showed very active granular cytoplasm.

In some sections of the vas deferens, the colloidal material shows small, circumscribed areas of dissolution (Figure 4.16), which are very similar in appearance to areas of dissolution found in mammalian thyroid follicular colloid (Cormack, 1987). The sperm release from the colloid may be a mechanism to facilitate sperm transfer when mature, but could also be a mechanism to facilitate sperm lysis and reabsorption by the lining cells of the vas deferens if copulation does not occur before the sperm have reached the end of their life, as occurs with spermatozoa in mammalian sperm ducts (Holstein, 1967). The significance of the variation in sperm density in the colloid is unknown. Sperm embedding in colloid may be associated with sperm nutrition and/or transport. Another possibility is that the colloid forms a reservoir of hormones (*e.g.*, androgen binding protein secreted by the sustentacular cells) and nutrients that are steadily released over time by dissolution.

4.4.4 Ovaries and Follicles

The ovary consists largely of follicles in various stages of development. The presence of enlarged follicles in all adult size categories suggests that reproduction continues in older geckos. The secretion of ovarian oestrogens, progesterones and androgens is controlled, at least partly, by the adenohypophyseal gonadotrophins (Yaron, 1985; Jones and Baxter, 1991). The reduction in hypophyseal gonadotrophin secretion *e.g.*, at the end of the reproductive season, should cause cessation of follicular yolking and possibly some reabsorption of yolk already produced. Involuting follicles probably all disintegrate and do not regrow (as is thought to occur in mammals, (Campbell and Reece, 1999)). The widespread occurrence of involuting (or atretic) follicles in many species, and in apparently healthy specimens in all seasons of the year, suggests that involution of follicles is probably normal (Hubert, 1985). Because follicular involution (atresia) accounts for the death of some follicles (Guraya, 1989), the number of follicles formed will exceed the number actually ovulated. The death of some follicles is linked to reduced hypophyseal follicle

stimulating hormone (Sinervo, 1994), possibly secondary to factors such as malnutrition, gravidity, end of reproductive season, or inclement weather (MacAvoy, 1976), but why it sometimes occurs in healthy females (including *Underwoodisaurus* and, probably, *Nephrurus* species) is not known. A possible advantage might be to reduce the numbers of older follicles, thereby reducing chance of gene mutation occurring.

4.4.5 Conclusions

Under captive seasonal conditions *N. amyae, N. asper, N. deleani, N. levis, U. milii* and *U. sphyrurus* will usually breed only in the spring and summer. Under captive conditions all these species also showed seasonal iteroparity. The data for museum specimens supports iteroparity in the wild, probably in all *Nephrurus* and *Underwoodisaurus* species, at least under favourable conditions. Possible exceptions are *U. sphyrurus* and *U. milii* in the higher and cooler parts of their ranges, where the summer season is relatively short.

The enlarged testes sometimes found in autumn in southern *Nephrurus* species, and possibly *U. milii*, suggests the possibility that autumn mating and sperm storage may occur with consequent earlier spring egg-laying. The limited evidence that mating occurs at this time or that over-winter sperm storage or egg retention occurs (Gow 1979; Couper, 1996) suggests that these phenomena may not be uncommon.

Although unseasonal weather is likely to interfere with the normal reproductive cycle, the reproductive characteristics of *Nephrurus* and *Underwoodisaurus* are able to cope with such interference. The large variations in reproductive cycles are an indication of considerable physiological plasticity, which may confer significant selective advantages because it enables these species to cope with sometimes-dramatic inter-annual climatic variations, as well as ecological and edaphic variations. *Nephrurus* and *Underwoodisaurus* have seasonal iteroparity, early sexual maturity in males, increased longevity, large egg size and sperm storage, all tending to counter any interruption of reproductive activity and thus contribute to increased fecundity. Hence, despite a small, fixed-clutch size, the fecundity may be comparable to other reptiles that reproduce only once a year but have a large clutch size or reduced longevity.

Chapter 5

Nephrurus and *Underwoodisaurus* Eggs

5.1 Introduction

5.1.1 Gecko Eggs and Egg Laying

Egg laying is the predominant mode of reproduction among geckos, with approximately 97 % of all gecko species laying eggs (Bartmann and Minuth, 1979; Kluge, 1993; Henkel and Schmidt, 1995; Hitchmough, 1997). All Australian geckos are oviparous (Cogger, 2000). Most oviparous reptiles produce a number of eggs that have been ovulated, fertilised and shelled almost simultaneously and then oviposited as a single clutch (Iverson and Ewert, 1991). A clutch may be similarly defined for geckos.

Nothing is known of egg laying in the wild by *Nephrurus* and *Underwoodisaurus* geckos, but it has been observed in captivity. Two published comments on egg-laying in *Nephrurus* geckos include a colour photo of *N. asper* at a burrow entrance 'laying eggs' (Henkel and Schmidt, 1995) and a description of *N. sheai* laying eggs into a small hole on the surface (Gow, 1979).

One important aspect of oviparity is clutch size (Smith and Fretwell, 1974; DeMarco, 1989; Shine and Greer, 1991), which is extremely variable among squamates in general, ranging from one in some mainly small skinks and geckos (Miller, 1984; Greer, 1989), to a maximum of over 70 in *Iguana iguana* (Fitch, 1985). In general, saurian clutch size varies as a function of female body size (Dunham, et al., 1988), although there are 24 distinct phylogenetic lineages in seven families of lizards (including some geckos) with invariant clutch size (Kluge, 1987; Shine and Greer, 1991). Clutch size is assumed to represent the optimum balance between size and number of offspring (Brockelman, 1975; Avery, 1975).

Clutch size in geckos is limited to one or two eggs (Zug, 1993; Henkel and Schmidt, 1995). The sphaerodactyline geckos (five genera and approximately 130 species) and about 20 of 723 gekkonine species lay only one egg per clutch (references in Appendix 7, Table A7.2).

The rest of the Gekkoninae, the egg-laying Diplodactylinae and the Eublepharinae geckos are all presumed to lay two eggs per clutch (Vitt, 1986; Szczerbak and Golubev, 1996; Greer, 1989; Doughty, 1997). An invariant clutch size is evidently an important part of the reproductive strategy of geckos.

Among Australian lizards there is an inverse relation between the degree of invariance of clutch size and the number of eggs in a clutch (Shine and Greer, 1991). All Australian gecko species normally lay two eggs per clutch except the gekkonine *Gehyra variegata* species complex geckos, which lay only one egg per clutch (Bustard, 1968b; Greer, 1989). Many authors have reported clutches of just one egg in a gecko species that usually lays two eggs (Appendix 7, Tables A7.3). However, in one gekkonine gecko, *Christinus marmoratus*, 71 % of 41 clutches consisted of two eggs, but this degree of bimodality of clutch size is possibly unique among geckos (Doughty and Thompson, 1998).

Relative clutch mass (RCM) (method 1, Greer, 1989) is an important indicator of maternal energetic output (Avery, 1975), but it has both ecological and genetic determinants (Vitt and Price, 1982). Thus, analysis of RCM of arid and mesic species should be useful in determining whether aridity affects egg size.

A possible difference in relative egg sizes of gekkonine and diplodactyline geckos has been suggested (Greer, 1989). If diplodactyline eggs are relatively larger than gekkonine eggs, the difference may be related to the differing reproductive strategies of the two subfamilies. Also, if absolute and relative egg sizes are affected by aridity and if gekkonine eggs are arid resistant, then the variance in gekkonine relative egg size would be less marked than among diplodactyline eggs. If arid *Nephrurus* geckos lay larger eggs than mesic *Underwoodisaurus* species it is possibly because larger eggs are more desiccation resistant than smaller eggs. That would also mean that relative pelvic width and body size in females would be greater among arid-adapted diplodactyline species than among mesic-dwelling diplodactyline, and possibly gekkonine species. If these selection pressures are exerted only on the females, it can also be predicted that sexual size dimorphism will be increased in favour of females in arid adapted species, unless other factors such as territoriality predominate. If only diplodactyline gecko eggs are affected by aridity, then the minimum size of diplodactyline eggs will be greater than that for sympatric gekkonine eggs.

Also, the minimum size of diplodactyline geckos will be larger than for gekkonine geckos in similar habitats. However, if arid diplodactyline geckos were always able to find suitably moist egg deposition sites, then the selection pressure would not exist and other factors such as phylogenetic or nutritional constraints would have to be invoked to explain any difference in relative egg sizes.

Among geckos there are two distinctly different types of eggshell, one group (Gekkoninae and Sphaerodactylinae) lays calcareous, rigid-shelled or endohydric eggs (Miller, 1984; Tracy and Snell, 1985) that are almost impervious to moisture loss, suggesting that these subfamilies are well adapted for reproduction in arid regions (or laying their eggs in dry microhabitats). The other group (Diplodactylinae, Eublepharinae and the Pygopodidae) lays flexible-shelled or ectohydric eggs (Bustard, 1968b; Deeming, 1988; Greer, 1989; Cogger, 2000), suggesting that they are is less well adapted to aridity. However, the contents of parchment-shelled reptilian eggs are hygroscopic, being able to absorb up to two or three times the initial egg weight of water (Badham, 1971; Packard, M. J. et al., 1980; Muth, 1981; Gutzke and Packard, G. C., 1987; Thompson, 1987; Packard, G. C. and Packard, M. J. 1988; Packard, G. C., 1991; Thompson and Russell, 1998). This ability to increase the size of the egg water reservoir may be important in providing increased resistance to desiccation. Thus, in this study I make a comparison of rigid-shelled gekkonine eggs and flexible-shelled diplodactyline eggs to determine the differences in reproductive strategy of the two groups.

The only data available for degree of embryonic development at oviposition in Australian geckos are for the gekkonine species *Christinus marmoratus* staged at 26.5 - 29 (Thompson and Russell, 1999b). The wide range for incubation time, both within and among Australian gecko species (Greer, 1989), suggests there is variation in stage of development at oviposition. Incubation period in Australian geckos ranges from 43 days at 30 °C for a small species, such as *Diplodactylus elderi* (Bustard, 1965a), to 273 days at a variable temperature (19-29 °C) for such large species as *Christinus guentheri* (Cogger, 1971). Even within a single species, the duration of incubation can vary dramatically from one population to another *e.g.,* a minimum of 75 days for a southern population of *Gehyra dubia* and a maximum of 148 days for a northern population, both incubated at 25 °C (Bustard, 1969).

Compared to most geckos *Nephrurus* and *Underwoodisaurus* geckos lay large, flexible-shelled eggs with two eggs per clutch (Gow, 1979; Delean and Harvey, 1984; Sameit, 1990; Ehmann, 1992; Annable, 1992; Bedford and Christian, 1993; Wagner and Lazik, 1996; Annable 1998).. With small clutch size, relatively more resources can be provided to any given egg to produce relatively large offspring. The limitation of egg size depends on egg shape, anatomy of the female reproductive system, abdominal volume and dimensions of pelvic canal (Iverson and Ewert, 1991). Whether the eggs are rigid-shelled or not may be irrelevant in this context, as both egg types are soft and pliable when first laid (Szczerbak and Golubev, 1996; Rogner, 1997).

Most flexible-shelled reptile eggs, increase in mass due to uptake of water during incubation (Dendy, 1899; Cunningham and Hurwitz, 1936; Gordon, 1960; Bustard, 1966; Packard, M. J., et al, 1980, 1982, 1985; Ar, 1988; Thompson, 1988; Ar, et al, 1990; Ackerman, 1991; Packard, G. C., 1991). The increase in mass of flexible-shelled reptile eggs during incubation varies from no increase in *N. deleani* (Delean and Harvey, 1984), 14 % in *N. asper* (Annable, 1992), 20 % in *N. deleani* (Annable, 1998), to approximately 300 % of initial mass in the agamid lizard *Pogona barbata* (as *Amphibolurus barbatus*) (Badham, 1971). In the early stages of embryonic development, before compartmentalisation of the egg by embryonic membranes, water uptake is entirely passive (Packard, 1991; Vleck, 1991), as confirmed by the similar initial rates of water uptake in dead or infertile eggs (personal observation). Later, the developing embryo has some influence over the rate of water absorption (Simkiss, 1991; Vleck, 1991). As the embryo develops, there is compartmentalisation of the egg together with thinning of the eggshell and increased superficial vascularity in the intermediate stages. The progressive excretion of urea into the allantois during embryonic development (Packard, G. C., et al, 1977) may also contribute to increasing water uptake by increasing the osmolality.

In this study, four species of *Nephrurus* (*N. amyae, N. asper, N. deleani, N. levis*) and both species of *Underwoodisaurus* (*U. milii and U. sphyrurus*) laid eggs in captivity. In addition, live egg data have been obtained from the literature or as personal communications for three more species (*N. laevissimus, N. sheai, N. stellatus*). There is no reason to suspect any major differences in reproduction in the two species for which no live egg data are available (*N. vertebralis* and *N. wheeleri*) compared to their congeners.

The major aim of this component of the study was to compare the physical characteristics of *Nephrurus* with *Underwoodisaurus* eggs, and then compare these eggs with those of other geckos to infer which characteristics are likely to be related to adaptation to aridity. Only *N. levis* and *U. milii* produced sufficient eggs to allow detailed analysis. Given the largely allopatric distributions of *N. levis* and *U. milii* and their differing bioclimatic requirements, they form a suitable pair of species for comparison of their egg characteristics. The major questions being addressed in this part of the study concern the fundamental reproductive characteristics of *Nephrurus* and *Underwoodisaurus* species:

1. What are the macroscopic, microscopic and ultrastructural characteristics of the eggs of *Nephrurus* and *Underwoodisaurus*?

2. What are the differences (size, shape, conductance and clutch mass) in the eggs of the arid *Nephrurus* species compared to the eggs of mesic to sub-mesic *Underwoodisaurus* species?

3. What effect does variation in temperature and water potential have on the incubation period of the eggs?

To address these questions, I compared the characteristics of *Nephrurus* and *Underwoodisaurus* eggs and eggshells and observed behaviours related to egg-laying and incubation. Similar observations were also made on some other related or outgroup geckos for comparison.

5.2 Methods

5.2.1 Egg Incubation

Nephrurus levis and *U. milii* were chosen as suitable representatives of their genera for study and were held in sufficient numbers to obtain multiple observations related to egg laying and egg characteristics (Appendix 6). As the time of oviposition approached, daily observations of about 5 - 15 minutes were made to detect changes in behaviour and to determine exactly when eggs were laid. By artificially adjusting the photoperiod using a time clock on the vivarium lighting, 'nocturnal' observations were largely made during the

late afternoon. A photographic far-red safelight, which had no noticeable effect on gecko behaviour, was used to facilitate observations. In over 90% of cases, the clutches were discovered the morning after being laid. The morning after was determined as the oviposition date from which all incubation periods were measured.

After oviposition, the females were weighed to the nearest 10 mg, the eggs were weighed to the nearest 0.1 mg and RCM was calculated as fresh wet clutch mass/post-oviposition maternal wet mass expressed as a percentage. As soon as possible after oviposition the nests were carefully excavated and the positions of the eggs recorded. Eggs were marked with a colour code near one end (away from presumed central position of embryo) using permanent marker pens while *in situ* then removed and measured using a digital calliper for length and breadth to the nearest 0.1mm. Care was taken to prevent substrate material attached to the eggshell from interfering with egg length and breadth measurements by carefully cleaning a small patch at each measuring point. Eggs were then cleaned of any loose sand, weighed and candled to determine fertility (not always possible with eggs covered in fine red sand), before transfer to incubation chambers.

Eggs were incubated half buried in 0.5 L semi-transparent plastic containers two-thirds filled with standardised, fine grain vermiculite substrate at water potentials of either -100 kPa or -450 kPa, and at temperatures of either 25 °C or 30 °C (*i.e.* four substrate conditions). Substrate water potentials were determined from a standard curve prepared for that batch of vermiculite using thermocouple psychrometry (Wescor HR33-T, microvolt meter and a C52 chamber) (Brown and Van Haveren, 1972). Seventeen eggs (*N. asper*, N = 3; *N. levis*, N = 2; *U. milii*, N = 12) were also incubated undisturbed at (varying) room temperature and approximately –250kPa, but decreasing water potential, and two at 35 °C. Eggs were chosen in order of laying so as to give equal numbers for each species in each substrate state. The eggs were half buried in the vermiculite to facilitate egg monitoring. The close-fitting container lids were not airtight and allowed some ventilation, but maintained high humidity. The eggs of each clutch were incubated either separately or in pairs in the containers, which were numbered and weighed. Slow evaporation was compensated by the addition of deionised water each week to make up the original mass. The two water potentials chosen were both expected to be within the normal range for incubation of flexible-shelled eggs (Packard, G. C., 1991). Eggs were incubated in 40 L

insulated oven-type incubators at either 25 ± 1 °C or 30 ± 1 °C so that the Q_{10} of embryonic development could be determined. Incubator temperatures were also monitored using a mercury-in-glass maximum and minimum thermometer.

5.2.2 Egg Size and Shape

The shapes of avian eggs are quantified using length, breadth and radii of curvature of the blunt and pointed ends of the egg (Preston, 1968; Hoyt, 1976; Maritz and Douglas, 1994). Determinations of the surface area of avian eggs using the formula ($A = 4.951.V^{0.666}$ where A = surface area and V = volume) give a maximum error of
3 % (Besch, et al, 1968; Paganelli, et al, 1974) irrespective of egg shape. Thus, shape does not have a great effect on the surface area-volume relationship of avian eggs.

Three major methods have been used to classify egg shapes and calculate symmetrical egg volumes from length and breadth measurements (Iverson and Ewert, 1991).

1. The cylinder formula, which assumes the egg, is cylindrical and symmetrical with a hemisphere on each end (similar to certain snake eggs) (Douglas, 1990; Iverson and Ewert, 1991)

$$\{V = \pi.(B^3/6) + \pi.(B^2/4)\ (L - B)\}$$

2. The ellipsoid formula (as in some lizard eggs) (Douglas, 1990; Iverson and Ewert, 1991)

$$\{V = \pi.L.B^2/6\}$$

(or $V = L\ B_1\ B_2/6$ where the egg is asymmetrical, Iverson and Ewert, 1991).

3. The bicone formula (for eggs with radial symmetry but not necessarily longitudinal symmetry, as in many avian eggs) (Preston, 1968; Tatum, 1975; Iverson and Ewert, 1991)

$$\{V = (\pi.L.B^2/6)\ (1 + 2/5C_2 + 3/36C_2^2)\}$$

where V = volume in microliters, B = breadth in mm, L = length in mm, and C_2 is the bicone value.

The bicone value is a measure of divergence from the ellipsoidal value of zero which is positive for egg ends with radii of curvature greater than for the ellipsoid of similar B and L values and negative for egg ends with radii less than for the equivalent ellipsoid. A range of bicone values is available for many avian eggs (Preston, 1969), but few reptilian eggs (dinosaurs (Seymour, 1979), turtles (Iverson and Ewert, 1991) and alligators (Deeming and Ferguson, 1991) but not geckos. A mean bicone value has to be calculated for each species prior to application of the formula (Preston, 1968; Iverson and Ewert, 1991). Some initial measurements of species-specific bicone values were made to determine egg volume, but all measurements for comparative purposes were made using the ellipsoid formula.

Bicone values (C_2) for 'pointedness' of eggs were determined in this study from the standard bicone formula used for avian eggs (Preston, 1968):

$$C_2 = (R_1 + R_2).(L/B^2) - 1$$

where R_b and R_p are the radii of curvature at the long axis poles, L is the length and B is the breadth of the egg.

Because reptile eggs in general do not have longitudinal asymmetry (Iverson and Ewert, 1991), the polar radii are close to identical and the formula becomes

$$C_2 = (2R.L/B^2) - 1$$

The eggs were photographed from a position perpendicular to the long axis to find the polar radii of curvature. The photographs were then enlarged to at least 100 mm diameter and a geometry compass was used to find the radius of curvature of each end of the egg. Egg length and breadth were measured to the nearest mm on the same photocopy enlargement using a ruler. Because the bicone value is a ratio there are no units and therefore it is not necessary to find the absolute values of the egg radii.

Eggs from available gekkonine species (*Cyrtodactylus louisiadensis, Gehyra variegata, Heteronotia binoei*) were also measured and weighed, as above, for comparative purposes.

Most of the limited data on diplodactyline eggs taken from the literature or personal communications were assumed or stated to be from recently laid eggs (Appendix 7, Table A7.1).

5.2.3 Relative Egg-size Index

A simple egg size index was devised for comparison of egg sizes among species where the index was calculated by:

$$I = (L + B).100/SVLmax$$

where I is the relative egg size index, L is the egg length, B is the egg breadth and SVL is the maximum female snout-vent length for the species. However because the egg-size index is not independent of SVL a second method of egg size comparison was used where the egg size was taken as length plus breadth.

The egg-size hypothesis that diplodactyline geckos lay relatively larger eggs than gekkonine geckos was tested by comparing egg size against maternal snout-vent length. The statistical power of the comparisons was improved in matching the two groups by including only species in the range of overlap in snout-vent lengths (for most of analyses), thus largely excluding any allometric interference. The egg and maternal SVL measurements of as many species as possible were otherwise included. Where multiple egg measurements were available from direct measurements or from the literature, the egg size index was calculated using the mean values for egg length and egg breadth. The egg size indices of arid *Nephrurus* and other diplodactyline gecko species were compared with the indices of comparable mesic *Underwoodisaurus* and other diplodactyline species ('arid' and 'mesic' as defined in Chapter 2).

Gecko egg and adult female size data were collected from the literature (Appendix 7, Table A7.1). The egg size index was plotted against SVL for both the rigid-shelled (gekkonine and sphaerodactyline) and flexible-shelled (diplodactyline and eublepharine) gecko species (Appendix 7, Table A7.1). A test for equality of variances was performed prior to analysis of covariance to compare the linear regression slopes and elevations (Sokal and Rohlf, 1995). Examination of the relationship between absolute egg size and maternal SVL was used to determine whether there is any significant relative size difference between

diplodactyline eggs and gekkonine eggs. A limited number of relative clutch mass (RCM) values were collated from the literature (Appendix 6, Table A6.9).

5.2.4 Eggshells

The shells of hatched eggs were weighed within 24 hours after hatching, washed, surplus water removed with filter paper and then weighed again to give mass of residue and wet mass of eggshell. Shells were measured while moist because they are difficult to measure accurately when dried as they become hard, irregular and brittle. The thickness of each eggshell was measured in at least ten different places to a precision of ± 0.1 μm using a standard SNK engineering micrometer with circular flat jaws. Each measurement was made with the same ratchet-controlled pressure. The mean thickness was then calculated for each egg (means presented as ± one STD). Percentage and proportion values were arcsine transformed prior to statistical analyses (Daniel, 1974).

Eggshell dry mass was determined by weighing after air-drying at room temperature for at least two days. Ash content of eggshell samples was determined using a Martin ST kiln with a Harco Electronics autocontroller. Fifteen eggshells (two *N. asper*, eight *N. levis*, five *U. milii*) were air dried in an incubator at 40 °C for several days and then weighed at room temperature. Dry eggshells were placed individually in weighed crucibles, reweighed and ashed in a muffle furnace at 500 °C for four hours. The ash content was calculated after again reweighing the crucibles when cool.

5.2.5 Electron Microscopy and Energy Dispersive Spectroscopy

Small pieces of air-dried gecko eggshell (*N. asper*, *N. levis*, *U. milii*) about 2-3 mm in diameter, were mounted on brass stubs, using a conductive carbon-based adhesive, to show internal and external surfaces as well as cross sections of the shell. Specimens of *G. variegata* eggshell were also treated as above for comparative purposes. The mounted specimens were sputter coated to approximately 5nm maximum thickness using a 20 nm spot size platinum electrode in an argon atmosphere at 5 x 10^{-2} mbar at 800 volts. The specimens were then viewed and photographed using a Philips SEM 505 scanning electron microscope. Small pieces of dried eggshell about one mm in diameter were vacuum embedded in epoxy resin and 5 μm sections were cut using a glass-knife microtome.

Sections were then mounted on copper grids for viewing using a Philips TEM 301. Energy dispersive spectrometry was performed using resin embedded eggshell pieces and the scanning was done across the cut face of the resin block using a Philips SEM 505. Energy dispersive spectrometry (EDS or X-ray scanning analysis) was also used to show the presence and location of both calcium and sulphur in the eggshell. Calcium and sulphur were chosen because they are both easily detected using EDS. Calcium (as calcite) was expected to be a major component of the outer part of the shell and sulphur was expected to be a major component (in cysteine and methionine) of the proteinaceous inner part of the shell. The presence of calcification in the eggshells was supported by light microscopic examination of the outer surface of pieces of eggshell, the scanning electron microscopy of both the surface and cross sections of pieces of eggshell, the transmission electron microscopy of cross sections of pieces of eggshell and by ashing of clean, dry shells.

5.2.6 Egg Mass, Density and Conductance Measurements

Eggs of *N. asper, N. amyae, N. deleani, N. levis, U. milii* and *U. sphyrurus* were weighed to the nearest mg. Egg density was found by weighing cleaned *N. levis* (N = 2) and *N. asper* (N = 2) eggs in air and under water of known temperature and density. An egg was suspended from the balance arm of a Sartorius null type analytic balance in a fine cotton sling and the density calculated from the weight of the egg in air and the volume of water displaced. Measurements of egg density were then compared with values obtained using the ellipsoidal volume formula. The density value obtained was further used to estimate the mass of eggs where only the linear measurements were given in the literature.

The conductance of *N. levis* and *U. milii* eggshells to H_2O and, therefore, to O_2 and CO_2, was measured by recording the desiccation rate of eggs exposed to an atmosphere of zero humidity for ten minutes, then calculating the loss rate per day. Shell conductance was calculated using the Fick diffusion equation

$$V_{H2O} = G_{H2O}(P_{H2O(outside)} - P_{H2O(inside)})$$

where V_{H2O} is the rate of water vapour loss ($mL.day^{-1}$), G_{H2O} is the water vapour conductance of the eggshell and P_{H2O} is the partial pressure of water vapour on each side of the shell. I assumed that the internal P_{H2O} at 25 °C = 23.8 torr (Lide, 1992) and the external

P_{H2O} was zero when using silica gel in a closed analytic balance. G_{H2O} was expressed as $mg.day^{-1}.torr^{-1}$. The room temperature was adjusted thermostatically overnight to that required for measurements, and the room humidity was calculated from a wet and dry bulb thermometer for ambient conditions.

To measure the initial loss rate, eggs were transferred to the balance with minimal delay (usually about 10-20 s). The weight of the egg was then recorded every half minute for two minutes, and then every minute for 10 minutes (or longer in some cases where the viability of the egg was not at risk). To find the maximum rates of water loss, weighing tests were done as above, but with four trays of desiccated silica gel inside the balance to reduce the humidity to zero. To find the eggshell conductance, the water loss rate was measured at two minutes from initial exposure to allow evaporation from the surface of the shell, but before significant change in submembrane P_{H2O}. Surface area specific conductance was calculated by dividing conductance by the calculated area of the egg. Gekkonine eggs of *Heteronotia binoei* (smallest available) and *Cyrtodactylus louisiadensis* (largest available) were weighed in a similar manner but, because their water loss rates were extremely low, the experiments were continued for 20-30 minutes. Immediately after completion of the experiment, the eggs were returned to the vermiculite incubation containers where any water losses were usually made up within a day.

5.3 Results

5.3.1 Egg-Laying Behaviour and Nest Sites

The complete egg-laying sequence was never observed, because it normally occurs underground. Nevertheless, numerous observations of parts of the sequence provide a broad overview of what takes place before, during and after oviposition.

The behaviour of the females of *N. asper*, *N. levis* and *U. milii* around the time of laying in captivity is variable. Some females dig several 'test' holes in the substrate before choosing a final laying site, whereas others dig only a single burrow. Digging in compacted sand involves the use of forelimbs and hindlimbs (Russell and Bauer, 1990). The manus is used to loosen sand as well as well as throw sand well past the tail tip in a single sweep action. The

pes is used to throw back loose sand within reach of the backward swing at least some of which having been loosened by the manus. The sequence of limb action began with alternating left and right manus or manus followed by pes on the same side, followed by manus then pes on the opposite side. The body is held clear of the substrate during digging.

The most common oviposition site is below or immediately adjacent to a rock or log at a depth of about 200 mm (the maximum depth provided in captivity). Other sites chosen included the corners and less commonly the sides of the vivaria or adjacent to a plant. When a nesting box was provided (Appendix 3), it was the preferred nesting site. The length of the nesting burrow was often difficult to determine unless the digging process was observed, but was usually 250-300 mm in length, with a terminal expanded part where the female could turn around and deposit the eggs. The site chosen was usually in a position where digging burrows was facilitated by compacted sand, although geckos attempted to dig in free-flowing sand by vigorously flinging the sand backwards using both forelimbs and hindlimbs.

In about 90 % of cases for all *N. amyae, N. asper, N. deleani, N. levis, U. milii* and *U. sphyrurus* clutches (N = 55) the two eggs were laid more or less parallel and narrowly separated by sand attached to the eggs and by loosely repacked sand. Eggs were always laid near the end of the nesting burrow, so that the eggs were in contact with undisturbed substrate. In the remaining clutches, the eggs were laid at various angles to each other or were in contact, but they were never stuck together. Normally the egg chamber is loosely but completely backfilled, but occasionally an air space was left (Figure 5.1). The eggs take several hours for the surface to dry out.

On one occasion a female *U. milii* was disturbed under a log while rolling her eggs (Figure 5.2), and several eggs showed evidence of scratch marks (*e.g.*, Figure 5.3). Nest construction by female *N. asper* is sometimes intermittent, with the female digging head first but occasionally turning around and coming to the mouth of the burrow, or even leaving it for a while, before resuming the digging or to oviposit. This behaviour was not observed in the other species. Backfilling of the nest in *N. asper* may also be intermittent, with the female returning the following night to pile more sand over the nest site. In this study the two eggs of a clutch were almost invariably laid within 24 hours of each other.

5.3.2 Embryonic Development

Female *Nephrurus* and *Underwoodisaurus* do not appear to be gravid until several days after mating (while the eggshell is being formed) and it is about three weeks or more (depending on temperature) before the eggs are laid (Annable, 1992). Candling of freshly laid eggs of *N. amyae, N. asper, N. deleani, N. levis* and *U. milii,* shows (unstaged) embryos approximately 2-4 mm in length. With most eggs, the area pellucida surrounded by the sinus terminalis is visible at the top of the egg within hours of laying. Initially the sinus terminalis is 8-14 mm in diameter, in some eggs it appears complete, while in others there was a small gap on the side opposite the proximal vitelline vessels. The sinus terminalis is invariably oval in shape, with the long axis always oriented perpendicularly to the long axis of the egg. The 'C' shaped embryo shadow is close to the centre of the sinus terminalis. The proximal vitelline vessels may or may not be visible, presumably depending on whether they are closely applied to the internal surface of the shell or not. An approximately circular area of slightly more translucent appearance lies within the embryonic disc, possibly due to presence of a watery, fluid-filled space *i.e.,* opacity due to yolk lipid droplets is absent. The sinus terminalis diameter increases to about 19 mm within a week of laying. The sinus terminalis develops up to 24 almost equally spaced radial capillaries that spread out away from the ring towards the ends and abembryonic pole of the egg. Most of these vessels atrophy and disappear, leaving mainly those passing towards the ends of the egg, where there is an increased surface area to mass ratio.

The early embryo always lies with its right side closely apposed to the inner shell surface (Greer, 1992). After one third of the incubation period, the eye pigment spots are visible through the shell, and movements may be detected in response to vibration or bright light. After two thirds of the incubation period, the body shadow is often distinctive. Also, a large clear patch near the embryo, which may represent the allantoic fluid, is visible. Both eggs of almost all clutches hatched within 24 hours of each other (for all gecko species observed) if incubated at the same temperature (see section 5.3.5). Incubation periods and associated data are given in the individual egg data tables (Tables 5.5 - 5.12).

Figure 5.1 Excavated nesting burrow of *N. levis* showing an air pocket above eggs.

Figure 5.2 Female *U. milii* disturbed under log while rolling her eggs immediately after oviposition. Note how the tail is curled around one of the eggs.

5.3.3 Egg Mass, Density and the Hydric Environment

Without exception, all diplodactyline eggs that hatched gained weight during incubation (Figure 5.4). However, one *N. asper* egg of initial weight 3.787 g collapsed after 104 days incubation at room temperature when it weighed 2.830 g (25.4 % loss). When opened the egg contained a live embryo, which died soon after. Also an *U. milii* egg initially weighing

1.609 g hatched after being desiccated to within 4 mg of the original mass just prior to hatching. Two clutches of eggs that were not discovered for some hours after laying in dry substrate were desiccated to the extent of having small dents in the shells. They failed to recover when rehydration was attempted, although the degree of desiccation was estimated at less than 10 %.

The mean increase in mass during incubation (in all incubation environments) of *Nephrurus* eggs was 24.7 ± 26.4 % (range 3.9 - 103.4 %, N = 21), which is significantly less than the mean increase for *U. milii* of 39.2 ± 20.3 % (range 12.9 – 95.7, N = 27), $t_{(1)46} = 2.322$, P = 0.012. The correlation between initial egg mass and hatchling mass for *U. milii* is positive but weak, ($R^2 = 0.252$, N = 31), also for *N. levis* ($R^2 = 0.348$, N = 17). The correlation between hatchling mass and maternal mass for *U. milii* is still weaker ($R^2 = 0.223$, N = 15), and for *N. levis* $R^2 = 0.007$ (N = 11).

There was no significant difference in the desiccation rates of eggs thickly covered in sand and those with little sand attached ($t_{(1)30} = 0.906$, P = 0.186), therefore the data were not separated. The mean initial rate of water loss from recently laid *U. milii* eggs with most of the sand removed was 1.42 ± 0.51 mL/day (N =10) at 22 °C and at 60-75 % relative humidity.

The increase in mass of six *U. milii* eggs incubated at 30 °C (34.9 %) and 26 eggs incubated at 25 °C (40.2 %) was not significant different. However, there was a significantly greater increase in mass of *U. milii* eggs incubated at -100 kPa compared to eggs incubated at -450 kPa ($t_{(1)14} = 3.35$, P = 0.002). The mean incubation period for

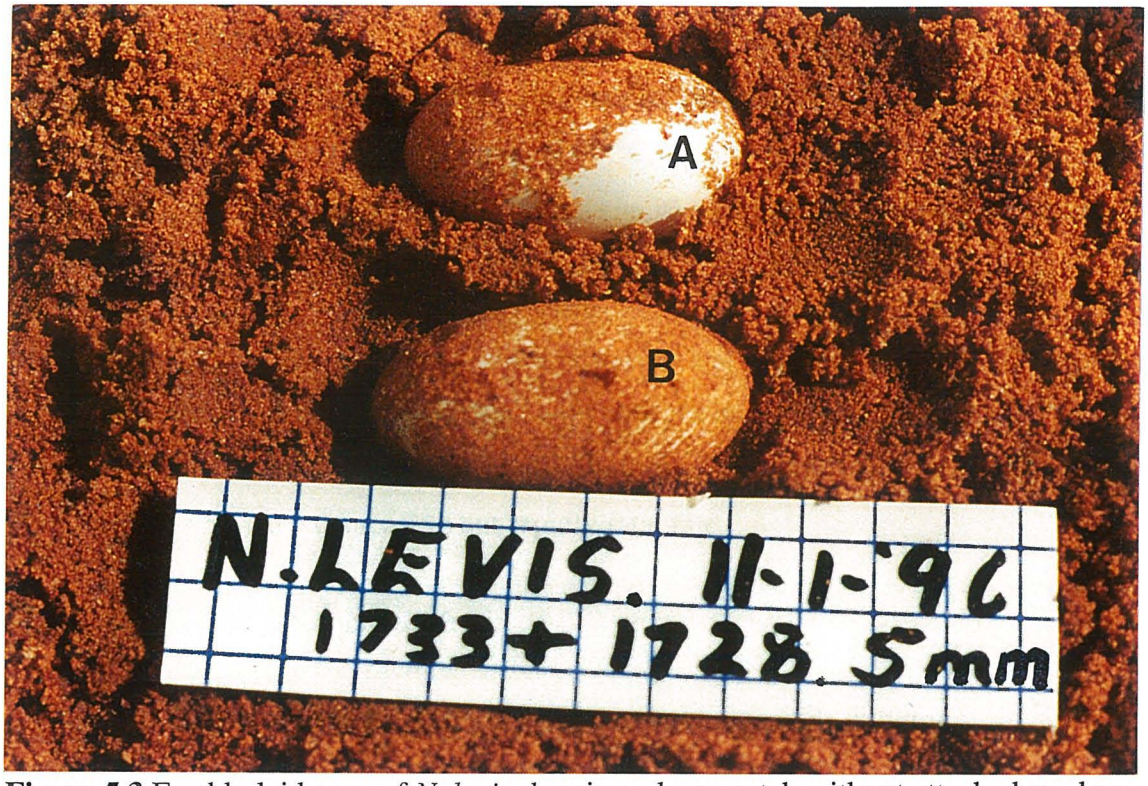

Figure 5.3 Freshly laid eggs of *N. levis* showing a large patch without attached sand on one egg (A) and scratch marks on the other (B). Fine red sand substrate is from the Simpson Desert region.

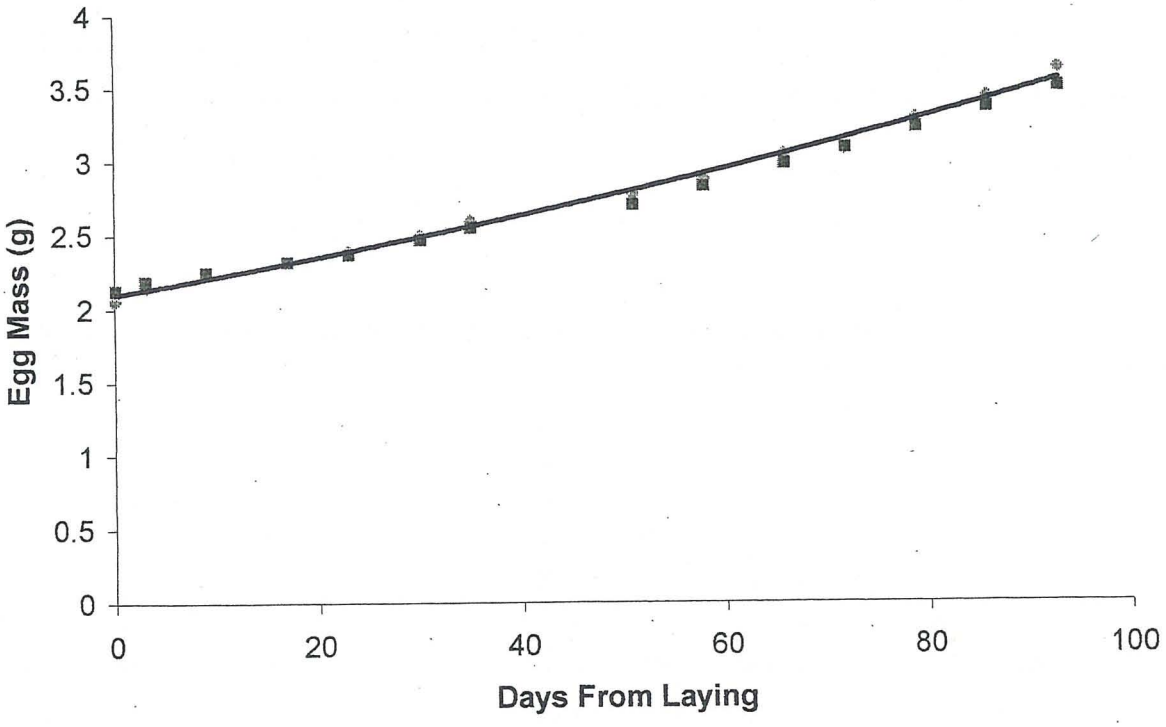

Figure 5.4 *Nephrurus levis* egg hydration during embryonic development. Two eggs (squares and diamonds, from one clutch) incubated at 25°C in -100kPa vermiculite substrate. Shows almost synchronous exponential increase in mass, fitted line given by $y = 2.097e^{0.0057x}$, $R^2 = 0.995$.

U. milii eggs incubated at 25 °C was significantly greater at -100 kPa than at -450 kPa ($t_{(1)10} = 2.17$, P = 0.0274). The mean *U. milii* incubation periods for moist and drier substrates were not significantly different at 30 °C, but N = 5 (*i.e.* more data needed to strengthen argument). In *N. levis* there was no significant difference in increase in egg mass at 25 °C compared to 30 °C nor at -100 kPa compared to -450 kPa.

Intact *Cyrtodactylus louisiadensis, Gehyra variegata* and *Heteronotia binoei* (Gekkonine) eggs were highly resistant to desiccation, with changes in mass during incubation on dry substrate of about 1 %. Diplodactyline eggs under similar conditions continue to lose water vapour (Figure 5.5) at a decreasing but rapidly fatal rate.

5.3.4 Egg Size and Shape

All *Nephrurus* and *Underwoodisaurus* eggs are prolate ellipsoids, ranging from near ellipsoidal to slightly hyperellipsoidal (positive bicone), and usually with radial and long axis symmetry. Mean and individual bicone values are all positive (hyperellipsoidal) in *N. levis* (N = 9) and in *U. milii* (N = 12) (Table 5.1). The means of bicone values for diplodactyline species (*N. asper, N. deleani, N. levis, U. milii, U. sphyrurus* and *Phyllurus platurus*) and gekkonine species eggs (*Gehyra variegata* and *Cyrtodactylus louisiadensis*) were not significantly different ($t_{(2)44} = 1.254$, P = 0.217).

Table 5.1 Gecko bicone values. STD = standard deviation, N = number of measurements.

	Nephrurus asper	*Nephrurus deleani*	*Nephrurus levis*	*Underwoodisaurus milii*	*Underwoodisaurus sphyrurus*	*Phyllurus platurus*	*Gehyra variegata*	*Cyrtodactylus louisiadensis*
Mean	0.467	0.384	0.278	0.316	0.186	0.464	0.222	0.080
STD	0.146	0.150	0.117	0.187	0.133	0.033	0.373	0.043
N	2	4	9	12	3	2	21	2

The mean relative size of flexible-shelled eggs (36 species of the Diplodactylinae and 6 species of the Eublepharinae) is significantly greater than among the species laying rigid-shelled eggs (27 species of the Sphaerodactylinae and 135 species of the Gekkoninae), although the slope is similar (Figure 5.6 and Appendix 7). A comparison of the 11 Australian gekkonine species and 36 Australian/ New Caledonian diplodactyline species shows significantly different slopes (P = 0.044) (Figure 5.7 and Appendix 7, Tables A7.1, A7.4 and A7.5). If the highly calcified eggs (personal observation) of the New Caledonian *Rhacodactylus* species are excluded from the analysis, then the slope for diplodactyline eggs is increased because the high leverage of the large outliers has been eliminated. Relatively larger eggs are laid by Australian diplodactyline geckos compared to Australian gekkonine geckos ($t_{2(33)} = 3.995$, P = <0.001) (Figure 5.8, Appendix 7, Tables A7.1, A7.4, A7.5). Although the slopes are significantly different, the relative egg size remains significantly greater among the Diplodactyline species compared to the gekkonine species. Data for 13 gekkonine species that lay single egg clutches (*Aristelliger barbouri, A. cochranae, A. praesignis, Asaccus elisae, A. gallagheri, Cyrtodactylus amictopholis, Gehyra variegata, Gymnodactylus geckoides, Hemidactylus bowringii, Pristurus flavipunctatus, Stenodactylus arabicus, Tropiocolotes persicus* and *T. steudneri*) (references in Appendix 7, Tables A7.1, A7.2) lay significantly larger eggs than gekkonine geckos laying two-egg clutches ($t_{(1)141} = 3.366$, P = < 0.001) and thus were excluded from the statistical analysis comparing diplodactyline and gekkonine relative egg sizes (all diplodactyline species lay two-egg clutches). The relative egg sizes are also significantly smaller among all gecko species laying two rigid-shelled eggs per clutch compared to those laying (two) flexible-shelled eggs ($t_{(1)157} = 2.201$, P = 0.0146) (Figure 5.6 and Appendix 7, Tables A7.1, A7.4 and A7.5). The relative egg size indices for rigid-shelled eggs (including single-egg clutches) (136 species from the Gekkoninae and 27 species from the Sphaerodactylinae) are significantly less than for flexible-shelled eggs (38 species from the Diplodactylinae, 6 species from the Eublepharinae) ($t_{(1)119} = -1.915$, P = 0.0289) (Figure 5.6, Appendix 7, Tables A7.1- A7.5). Arboreal diplodactylines have smaller eggs than terrestrial species ($t_{(2)22} = 1.947$, P = 0.032), but data were inadequate for comparison of arboreal and terrestrial gekkonine eggs.

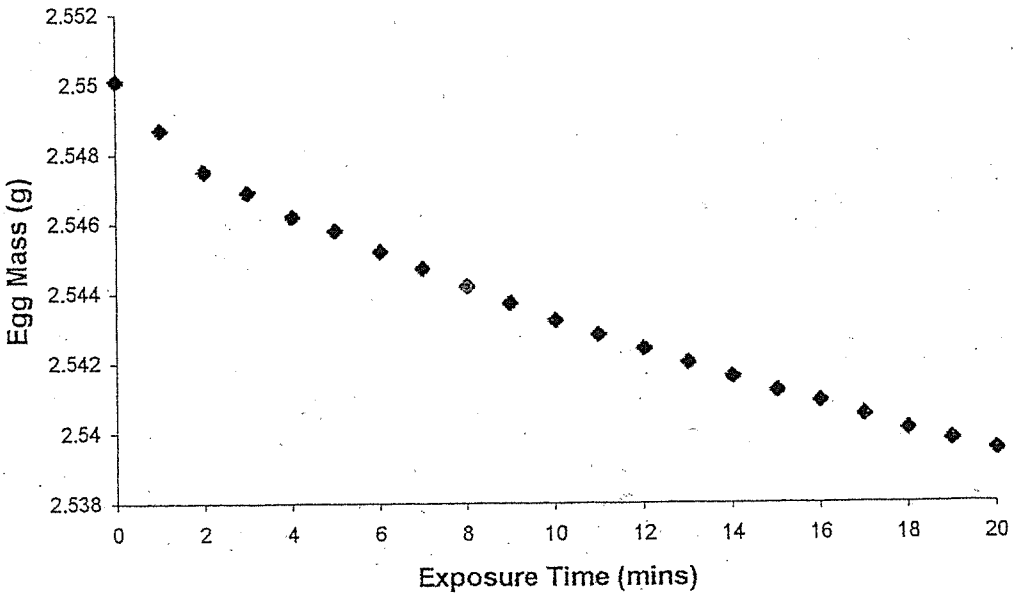

Figure 5.5 The typical changes in mass of a *N. levis* egg (N = 1) while being exposed to room air at approximately 20 °C and 70 % relative humidity. The egg loses weight continuously until embryonic death occurs, unless restored to a high humidity atmosphere when the losses may be regained within a few hours or sometimes days.

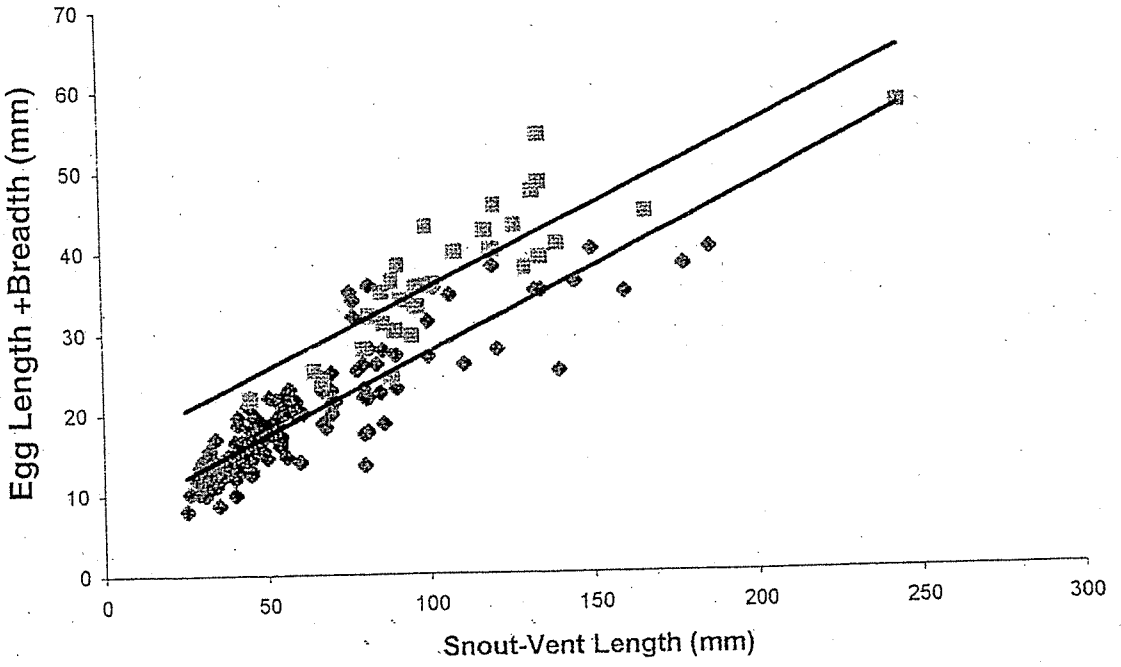

Figure 5.6 Comparison of relative egg sizes for geckos laying flexible-shelled eggs (Diplodactylinae and Eublepharinae) (squares, N = 42 species, y = 0.200x + 15.50, upper regression line) with those laying rigid-shelled eggs (Gekkoninae and Sphaerodactylinae) (diamonds, N = 162 species, y = 0.201x + 7.50, lower regression line) showing difference in egg size relative to maximum maternal size (references in Appendix 7).

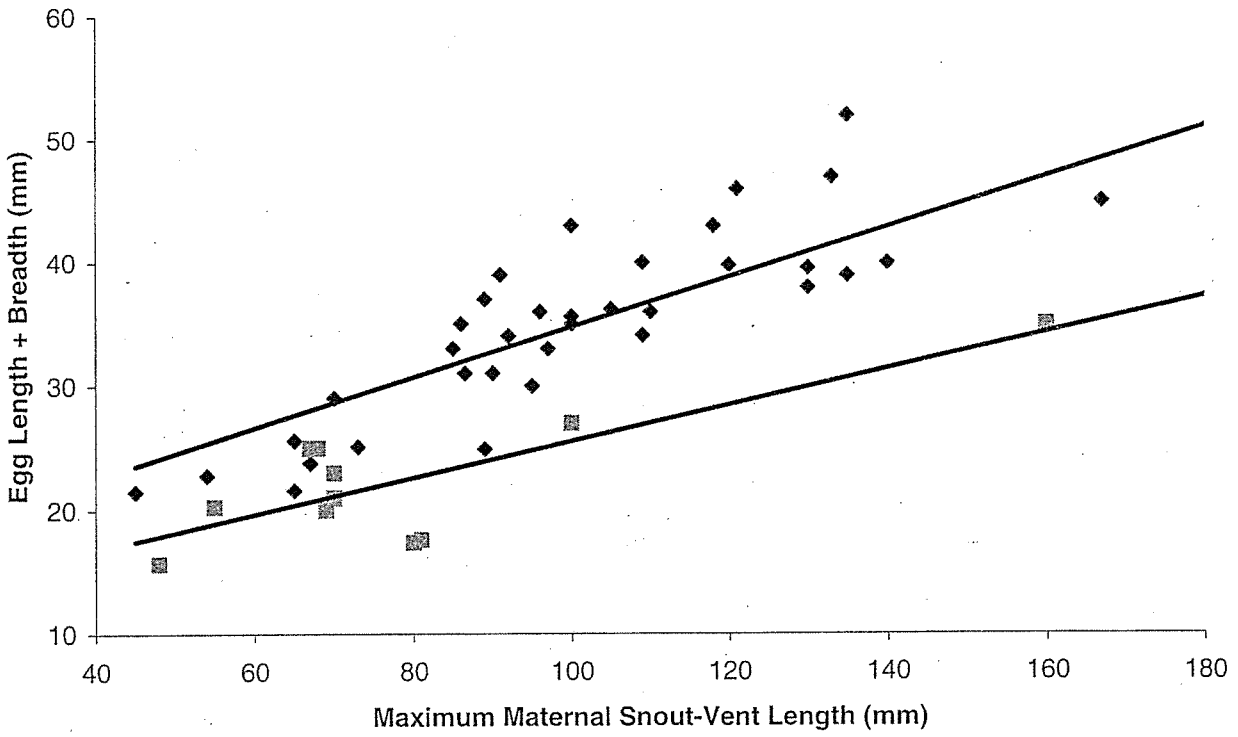

Figure 5.7 A comparison of Australian gekkonine with all diplodactyline species eggs including several large species that lay highly calcified eggs. The regression lines are given by y = 0.204x + 14.37 (N = 36 diplodactylines, diamonds, upper line) and y = 0.147x + 10.87 (N = 11 gekkonines, squares, lower line) which are significantly different in slope ($t_{2(33)} = 3.002$, P = 0.004) (references in Appendix 7).

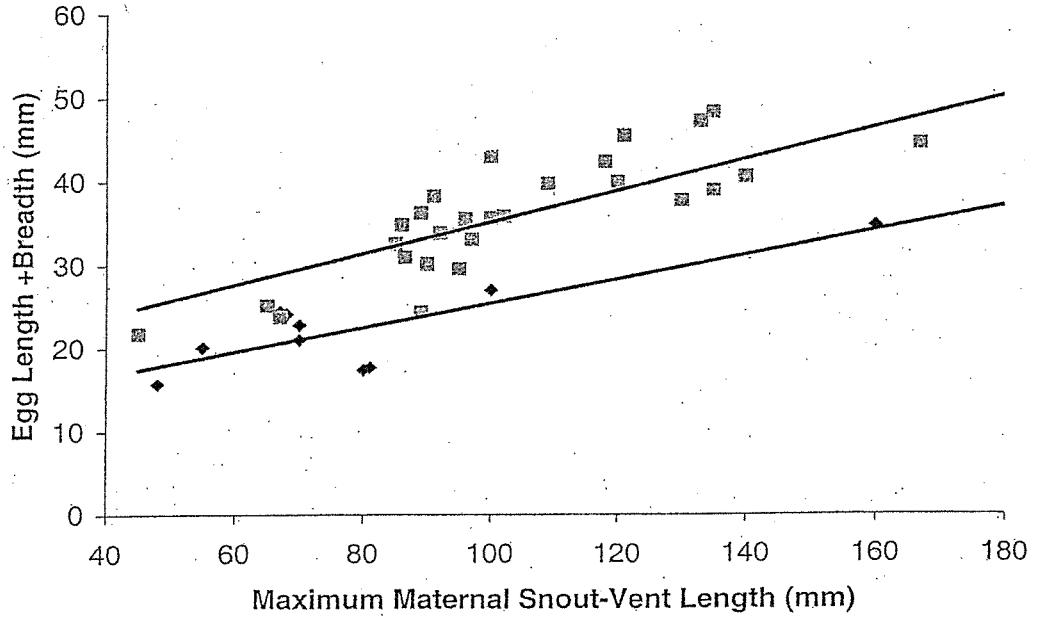

Figure 5.8 Australian diplodactyline geckos (squares, N = 24, y = 0.256x + 10.5, upper regression line) lay relatively larger eggs, compared to maternal size, than Australian gekkonine geckos (diamonds, N = 10, y = 0.150x + 10.8, lower regression line). The lines are significantly different in slope (P<0.05) (references in Appendix 7).

During incubation egg surface area increased by approximately 18 % in *U. milii* (12.6 % in *N. levis*) taking into account that egg breadth increases more than egg length. The mean increase in surface area of fertile *Nephrurus* eggs (11.5 % ± 10.1, N = 22) is not significantly different from the increase in *U. milii* eggs (18.0 % ± 10.1, N = 37). Stretching of the eggshell during incubation must be evenly distributed over the surface of the egg to prevent aneurysm formation. The stretching will increase surface area as well as area-specific conductance to oxygen, carbon dioxide and water vapour, e.g., if the surface area increases by 18% and thins by 18% the total increase in conductance is approximately 39 % (if initial conductance is 100 % then, after stretching, the conductance becomes 100 % + 18 % + 18 % of 118 % = 139 %).

The size of gecko eggs among species is correlated with the maternal SVL ($R^2 = 0.802$, N = 205 species). Although the mean maternal sizes of gekkonine, sphaerodactyline, diplodactyline and eublepharine geckos differ widely (Appendix 7, Table A7.5), the subfamily mean relative egg size index ranges only from 33.9 % (Gekkoninae) to 37.3 % (Sphaerodactylinae). Also the correlations of egg size with maternal size are both high for the diplodactyline and for gekkonine species ($R^2 = 0.755$ and 0.763 respectively) (Figure 5.6).

Relative egg size of arid-adapted diplodactylines (available data includes *Nephrurus* species, *Diplodactylus galeatus, D. elderi*) is significantly larger than non-arid diplodactyline species (Appendix 7) ($t_{2(28)} = 2.917$, P = 0.003). Similarly, the relative size of *Nephrurus* species eggs are larger than *Underwoodisaurus* species eggs ($t_{(2)8} = 2.430$, P = 0.020). Also, the egg size indices of the six (absolutely) largest arid-adapted diplodactylines are significantly smaller than the six smallest arid-adapted species (Appendix 7, Table A7) ($t_{2(10)} = 3.240$, P = 0.004). The largest gekkonine species also produce relatively smaller eggs than the smaller gekkonines. The relative egg size index for *N. levis* is close to the maximum for the genus at 39.1 % (the largest is *N. stellatus* at 40.6 %) and is significantly greater than for *U. milii* at 32.6 % (including two and one egg clutches) ($t_{(1)122} = 9.638$, P = <0.0001). The adult females of *N. levis* and *U. milii* are very similar in size, but the eggs of *N. levis* are significantly larger ($t_{(1)124} = 7.819$, P = <0.0001).

The mean length-breadth ratio (LBR) (or coefficient of elongation) for the eggs of 205 species of geckos is 1.41 (references in Appendix 7). However, there are two major divisions, the rigid-shelled eggs (Gekkoninae and Sphaerodactylinae) with a mean ratio of 1.27 ± 0.140 (N = 163) and the flexible-shelled eggs (Diplodactylinae and Eublepharinae) with a mean ratio of 1.86 ± 0.210 (N = 43) (Table 5.2 and Appendix 7).

Table 5.2 Mean egg length/breadth ratio in gecko subfamilies and subfamily groups. (STD = standard deviation, N = number of species).

TAXON	MEAN	RANGE	STD	N
Gekkonidae	1.40	1.0-2.55	0.288	206
Gekkoninae	1.26	1.0-1.67	0.144	135
Diplodactylinae	1.87	1.53-2.55	0.222	37
Sphaerodactylinae	1.33	1.06-1.50	0.105	28
Eublepharinae	1.86	1.67-2.08	0.136	6
Gekkoninae + Sphaerodactylinae	1.28	1.0-1.71	0.147	163
Diplodactylinae + Eublepharinae	1.87	1.60-2.5	0.211	43
Australian Diplodactylinae	1.85	1.6-2.4	0.186	27
Australian Gekkoninae	1.17	1.1-1.2	0.079	11
Nephrurus	1.83	1.73-1.93	0.066	7
Underwoodisaurus	1.94	1.89-1.99	NA	2

Within the rigid-shelled eggs, the sphaerodactyline eggs are more elongate (mean LBR = 1.33) than the gekkonine eggs (mean LBR = 1.26) ($t_{(1)146}$ = -1.668, P = 0.049). The LBRs for eublepharine and diplodactyline gecko eggs are not significantly different ($t_{(2)38}$ = 0.048, P = 0.962). The length/breadth ratio is significantly less among all rigid-shelled eggs compared to flexible-shelled eggs ($t_{(1)203}$ = 18.5, P = <0.0001). The mean LBR for all gekkonine eggs of 1.26 is significantly less than the LBR for the eggs of diplodactyline species ($t_{(1)156}$ = 16.96, P = <0.0001). The mean value for the LBR in *U. milii* is 1.89 ± 0.10 (N = 46), which is significantly greater than for all *Nephrurus* egg measurements ($t_{(1)176}$ =

5.91, P = <0.0001). The mean LBR for sphaerodactyline geckos is 1.33, which is also significantly less than for diplodactyline eggs ($t_{(1)60}$ = -11.99, P = <0.0001). The LBR for the eggs of Australian gekkonine species is 1.18 (N = 11) which is significantly less than for the non-Australian gekkonine species which is 1.28 ($t_{(1)119}$ = -1.872, P = 0.032).

5.3.5 Eggshells

The mean wet shell thickness of *N. levis* eggs (95.6 μm) is 14 % thicker than for *U. milii* eggshells (Table 5.3). The dry shell mass is 9.1 % greater for *N. levis* eggshells. The large standard deviations demonstrate a real variation in shell thickness, but may also reflect the difficulty of thoroughly cleaning eggshells without removing some of the shell material. For comparison, the mean electron microscopic measurement of shell thickness of *N. levis* eggs was 65.8 μm, range 49-81 μm (N = 3 eggshells). The eggshell dry mass per cm^2 of eggshell surface is not significantly different in *N. levis* and *U. milii* (P = > 0.05).

Table 5.3 Wet eggshell thickness (μm) and dry mass (mg). STD = standard deviation, N = number of measurements.

SPECIES	MEAN WET SHELL THICKNESS (μm)	STD	MEAN DRY SHELL MASS (mg)	STD	DRY MASS /AREA (mg/cm^2)	N
N. levis	95.6	68.9	45.7	23.7	4.27	8
U. milii	83.8	25.9	41.9	21.6	4.67	12

The flexible eggshells of *Nephrurus* have an outer layer containing calcium. Macroscopically, the clean, healthy eggshell is white, although some eggs have a patch (or patches) of incompletely calcified shell that is more translucent (Figure 5.9). Under a light microscope a thin, moderately homogeneous outer layer of opaque white material covers the deeper fibrous layer. The surface layer is semifluid when the egg is laid, as confirmed by the meniscus which forms adjacent to grains of sand adhering to the shell; the meniscus remains after the shell has dried (Figure 5.10). The eggshell of *U. milii* and *N. asper* are approximately half ash compared to about one third for *N. levis* (Table 5.4). The difference between *N. levis* and *U. milii* is significant, ($t_{(2)11}$ = 3.52, P = 0.005).

Table 5.4 Egg-shell ashing results. STD = standard deviation, N = number of measurements.

SPECIES	MEAN EGGSHELL MASS (mg)	MEAN ASH (mg)	ASH %	STD	N
N. asper	-	-	53.6	1.8	2
N. levis	45.7	1.60	35.1	7.5	8
U. milii	41.9	2.02	48.2	4.4	5

The calcium carbonate in the eggshell of *N. levis* consists of fine crystalline particles, which are visible under transmission (Figure 5.11) and scanning electron microscopy (Figure 5.12). The inner boundary layer of the shell is very thin and smooth with numerous small elevations and depressions, some of which (at least) are related to the underlying protein fibrils (Figure 5.13). No holes or pores were seen in the inner boundary layer at magnifications up to x 6,500. The outer surface of the shell has a few scattered holes of irregular size and structure (Figures 5.14, 5.15). EDS of shell sections shows two layers of high concentration of calcium in the outer shell (Figure 5.16) and a high concentration of sulphur in the inner fibrous (proteinaceous) region of the shell. In tangential section (Figure 5.17), the proteinaceous fibrils of the shell membrane are frequently seen as multiple layers (probably >8). Many of the layers are oriented approximately perpendicularly to each other. No fused fibrils or organic globules associated with the protein fibrils were seen.

5.3.6 Single-egg Clutches

Some gecko species that usually produce two-egg clutches occasionally lay just one egg per clutch; two out of 12 clutches (16.7 %) of *N. asper* eggs, five out of 24 clutches (20.8 %) of *N. levis* and eight out of 44 clutches (18.2 %) of *U. milii* eggs laid in captivity were one-egg clutches. Of 12 eggs laid by a single *N. asper*, the two largest eggs (4.15 and 4.78 g) were both single egg clutches laid late in the summer (the average mass of the other eggs was 3.53 ± 0.458 g). The mean mass of individual eggs laid in one-egg clutches for all the study species was significantly less than the mass for two-egg clutches ($t_{(1)10} = 2.389$, P = .0190).

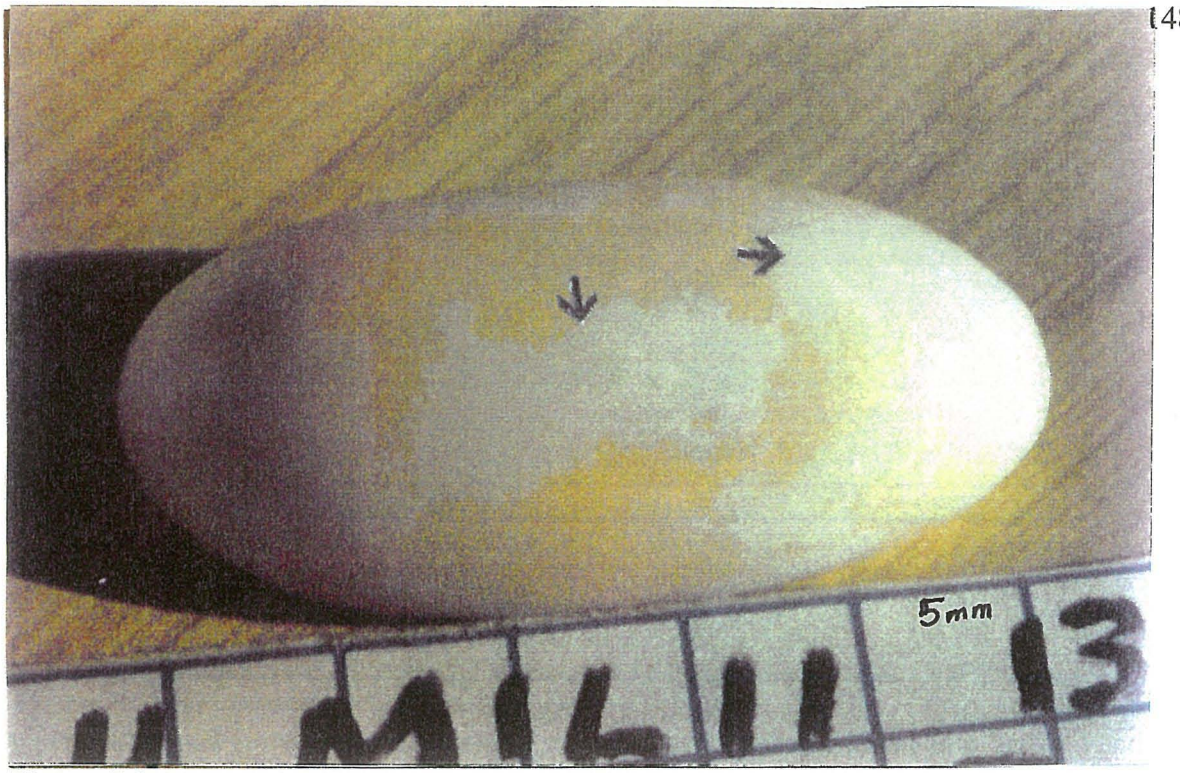

Figure 5.9 A partially calcified *U. milii* egg showing patchy distribution of calcium salts (pale areas, arrows) suggesting that the egg is not rotated by oviduct during calcium deposition.

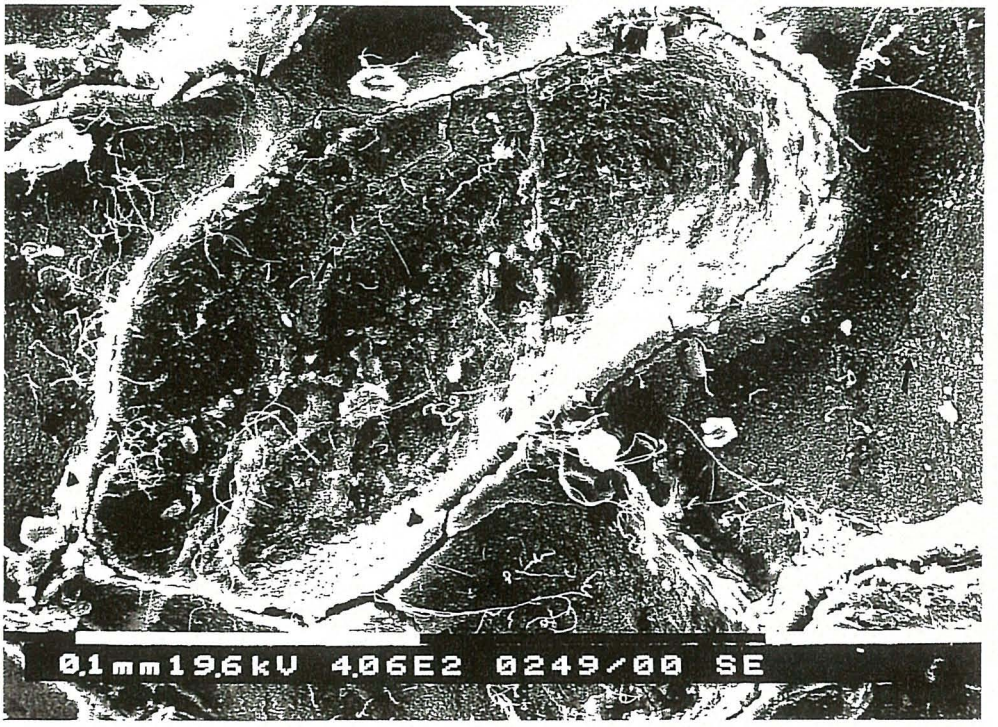

Figure 5.10 Scanning electron micrograph showing impression left by a grain of sand on the surface of an egg of *N. asper*. A meniscus (arrowheads) is seen around the margin of contact of the egg with the sand grain. Some of the cracks in the shell surface may be exaggerated or caused by the preparative process. Occasional small holes are seen in the surface (arrows). The fibres are fungal hyphae. Scale bar = 0.1 mm.

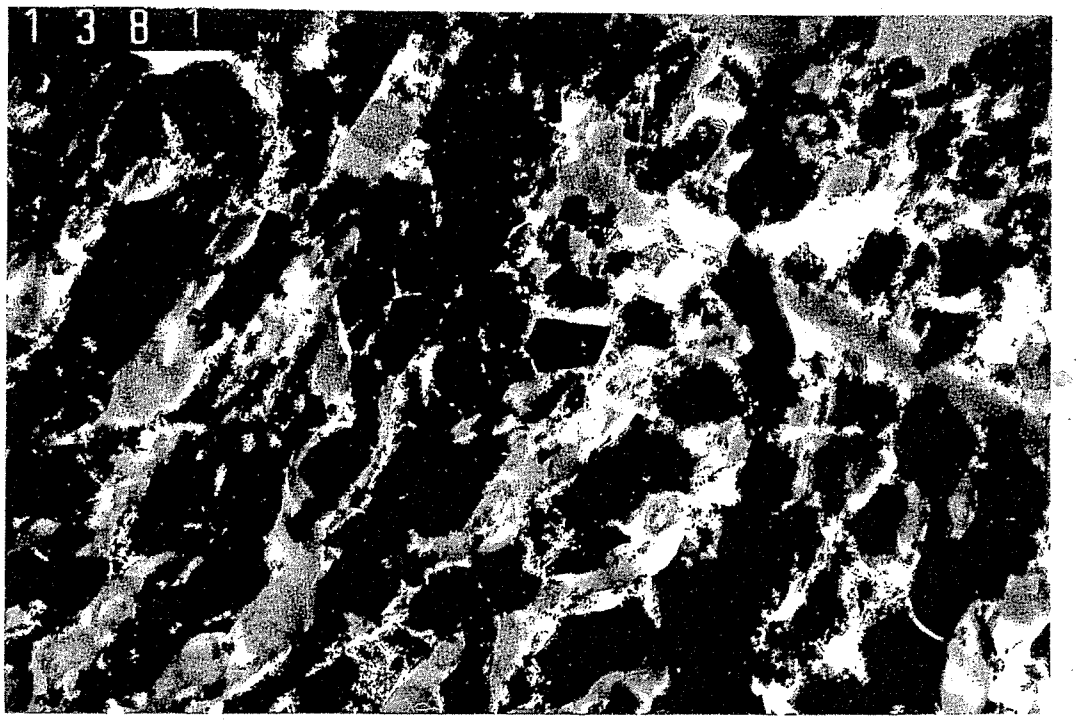

Figure 5.11 Transmission electron micrograph of *N. levis* eggshell near junction of outer calcite and inner fibrous layers showing open structure of shell fibrils (grey, arrows) and scattered crystals of calcite (black). The minute amorphous particles (p) on the surfaces of crystals and fibrils may be dried remains of the egg fluid. Scale bar = 1.0μm.

Figure 5.12 Fractured (*i.e.*, not cut) section through an eggshell of *N. levis*, showing outer zone of calcite crystals (top left), middle zone of protein fibrils (shell membrane or membrana testacea) and smooth inner boundary layer (lower right). Some small calcite crystals are laying on the outer surface which also shows a crack. Attached to the fibrils is a small amount of amorphous material possibly derived from the fluid matrix of the fibrous layer in life.

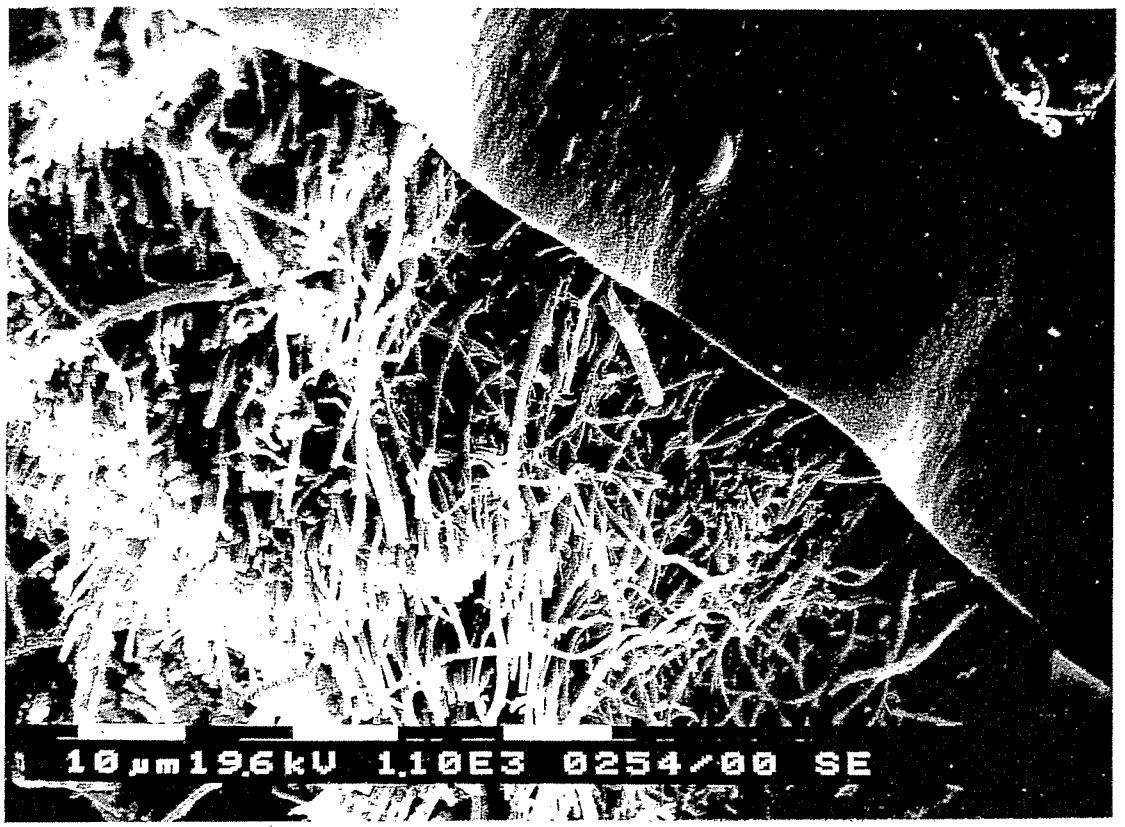

Figure 5.13 The inner boundary layer (top right) of the *N. levis* eggshell is very thin (approximately 0.02µm thick) and continuous, it is without perforations and acellular.

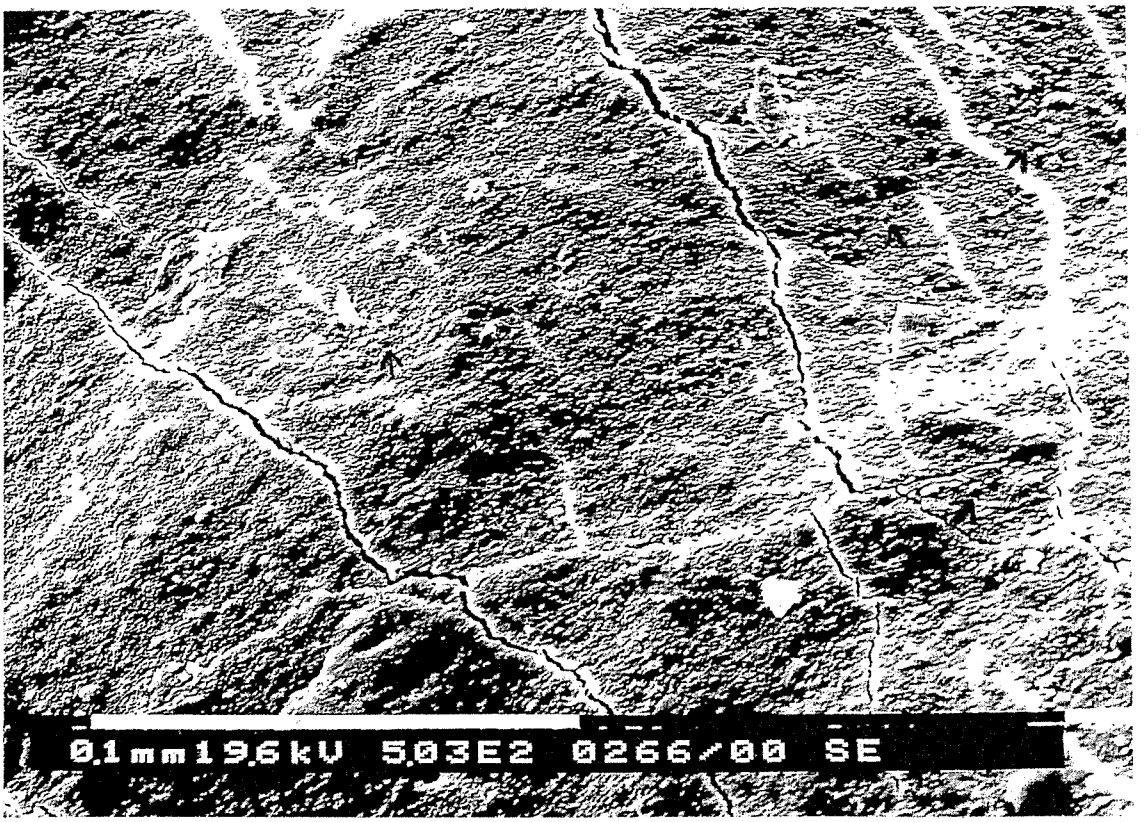

Figure 5.14 The outer shell surface of *N. levis* eggshell shows occasional and irregular perforations (arrows), some of which reach as far as the fibrous layer.

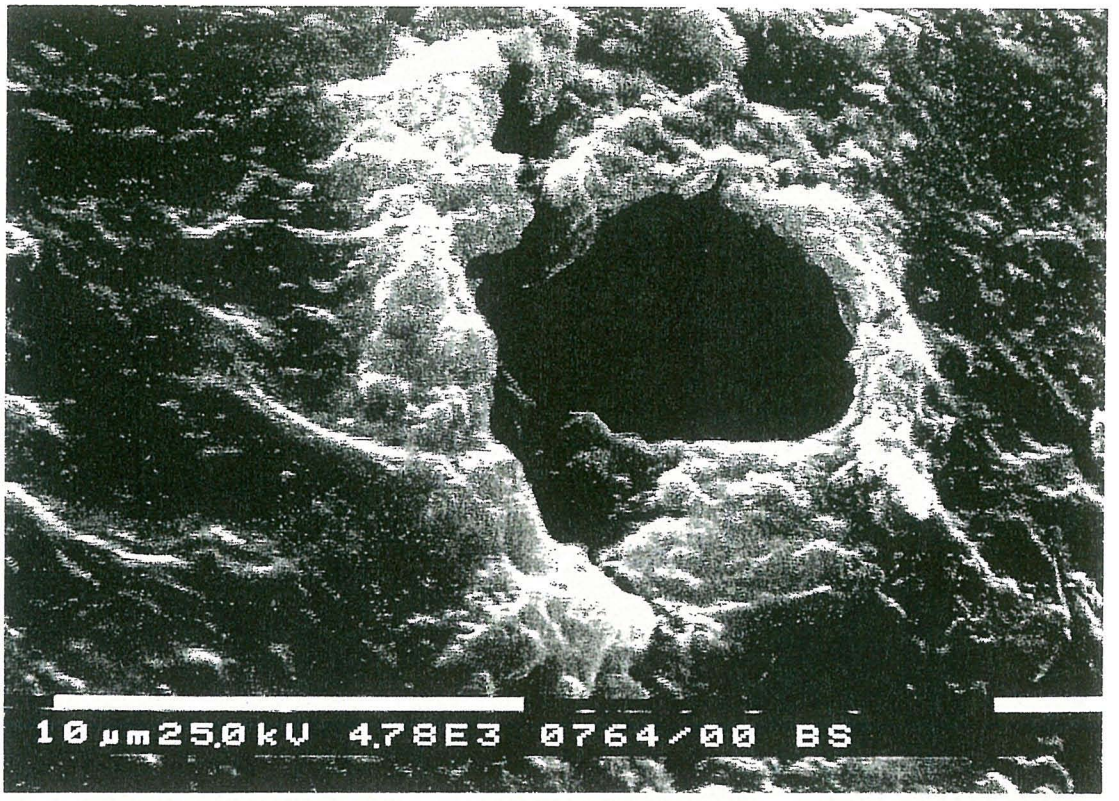

Figure 5.15 The outer shell surface of *N. levis* eggshell at higher magnification, the shell pores are seen as irregular in outline and with irregular internal structure.

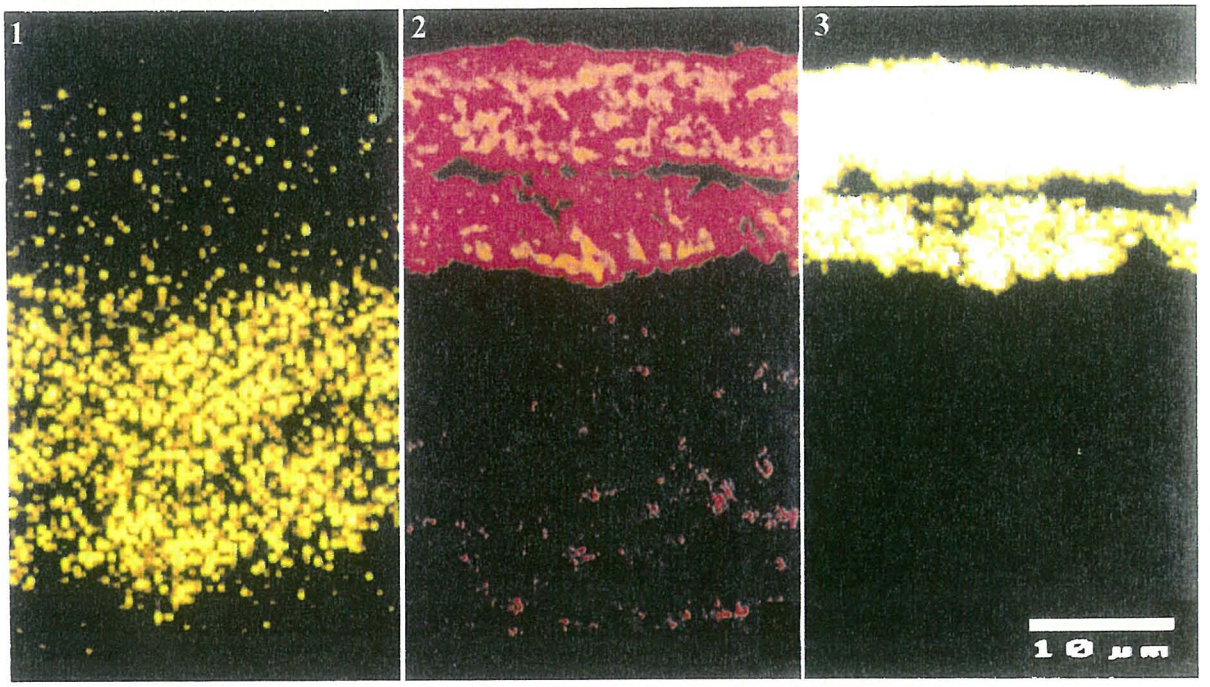

Figure 5.16 Three views of a single cut section of *N. asper* eggshell. 1. An EDS scan shows a predominance of sulphur in the fibrous (protein) layer (below). 2. A secondary electron image shows up the split calcium layer (above). 3. A second EDS scan shows that virtually all the calcium is in the outer layers of the shell (top).

Figure 5.17 A tangential view of the fibrous layer of a *N. levis* eggshell with the outer calcite layer removed shows structure and arrangement of fibrils. The fibrils vary in diameter from approximately 0.18 µm to 0.90 µm. Many of the fibrils are arranged approximately perpendicularly to each other and show approximately nine layers or sequential changes in direction.

5.3.7 Eggs of *Nephrurus* species

Nephrurus amyae

Measurements of six clutches of *N. amyae* eggs, all of two eggs, were analysed as a group. The largest of all *Nephrurus* species lays the largest eggs and has the largest hatchlings (Table 5.5). The relative clutch mass and egg size index are low among *Nephrurus* species, but larger than in *U. milii*. The increase in egg surface area during embryonic development is less than in all other *Nephrurus* and *Underwoodisaurus* gecko eggs. Detailed analyses are restricted because of small sample sizes.

Table 5.5 Egg data for *N. amyae**. STD = standard deviation, N = number of measurements.

CHARACTER	MEAN	RANGE	STD	N
Initial length (mm)	30.8	28-37	2.54	12
Initial breadth (mm)	17.3	16.1-18.1	0.724	12
Initial wet mass (g)	5.27	4.66-5.80	0.370	10
Length/breadth ratio	1.89	1.68-2.30	0.242	12
Length + breadth as % SVL	35.1	32.3-38.8	1.73	12
Volume (mL)	4.83	3.90-5.29	0.425	12
Density (g/mL)	1.06	0.965-1.16	0.099	4
Maximum mass (g)	5.63	5.12-6.14	NA	2
Mass increase %	18.1	9.87-26.3	NA	2
Initial area (cm^2)	16.6	14.3-18.7	1.74	6
Maximum area (cm^2)	17.3	17.0-17.5	NA	2
Surface area increase %	2.09	1.73-1.44	NA	2
Maximum length (mm)	31.3	30.8-31.7	NA	2
Length increase %	0.639	0.00-1.28	NA	2
Maximum breadth (mm)	17.6	17.6	NA	2
Breadth increase %	1.44	1.15-1.73	NA	2
Relative clutch mass %	28.0	24.8-32.3	3.86	3
Incubation at 30°C (days)	56	NA	NA	1
Hatchling SVL (mm)	52.6	NA	NA	1
Hatchling tail (mm)	11.8	NA	NA	1
Hatchling mass (g)	4.12	NA	NA	1
HM as % initial egg mass	88.5	NA	NA	1
Mean clutch size	2.0			

* Includes published measurements from one clutch (Bedford and Christian, 1993) and four clutches from Robert Porter, personal communication (no eggs hatched as part of this study). STD = standard deviation, N = number of eggs, NA = not applicable.

Nephrurus asper

The 20 *N. asper* eggs analysed come from 9 clutches of two eggs and two clutches of one egg. (Table 5.6). The eggs and hatchlings are large compared to most other species. The hatching rate was 86 %. Eggs incubated at room temperature took up to 130 days to hatch.

Table 5.6 Egg Data for *N. asper**. STD = standard deviation, N = number of measurements.

EGG CHARACTER	MEAN	RANGE	STD	N
Initial length (mm)	26.7	19.3-29.7	2.50	26
Initial breadth (mm)	15.3	12.9-16.7	0.985	26
Length/breadth ratio	1.73	1.50-1.83	0.074	26
Length + breadth as % SVL	35.5	27.3-39.3	2.92	18
Volume (mL)	3.31	1.68-4.34	0.663	18
Initial wet mass (g)	3.69	2.56-4.78	0.593	20
Density (g/mL)	1.05	0.764-1.15	0.113	20
Maximum mass (g)	4.33	3.90-4.78	0.325	9
Mass increase %	23.0	4.23-40.2	10.8	8
Initial surface area (cm^2)	13.5	11.2-15.6	1.19	20
Maximum area (cm^2)	14.2	13.2-15.6	0.974	7
Increase in surface area %	7.91	-0.11-19.7	8.51	7
Maximum length (mm)	27.0	24.9-29.7	1.57	7
Length increase %	0.189	-1.10-0.810	0.756	7
Maximum breadth (mm)	16.8	16.1-18.4	0.763	7
Breadth increase %	8.08	0.00-18.8	8.03	7
Relative clutch mass %	29.3	27.8-30.3	1.36	4
Incubation period at RT (days)	108	79-130	16.9	10
Hatchling SVL (mm)	46.1	44.0-48.0	1.63	4
Hatchling tail (mm)	10.8	10.0-12.0	0.957	4
Hatchling mass (g)	2.65	2.07-3.20	0.375	8
Hatchling mass as % egg mass	73.7	65.0-91.0	9.03	7
Mean clutch size	1.83			

* Includes published measurements of four clutches (Sameit, 1990; Couper, 1996). RT = room temperature (varying between 20.0 - 34.5°C).

Nephrurus deleani

Five clutches of *N. deleani* eggs (two eggs each) were analysed. *Nephrurus deleani* geckos have the largest RCM and second largest relative egg size among *Nephrurus* and *Underwoodisaurus* geckos (Table 5.7).

Table 5.7 Egg data for *N. deleani.* STD = standard deviation, N = number of measurements.

CHARACTER	MEAN	RANGE	STD	N
Initial length (mm)	23.5	20.7-25.4	1.54	10
Initial breadth (mm)	12.8	11.4-13.5	0.612	10
Length/breadth ratio	1.83	1.66-2.04	0.129	10
Length + breadth as % SVL	40.3	37.3-42.9	2.13	10
Volume (mL)	1.96	1.59-2.34	0.274	10
Initial wet mass (g)	2.18	1.71-2.52	0.297	8
Density (g/mL)	1.07	0.988-1.28	0.087	8
Maximum mass (g)	2.49	2.35-2.69	0.120	8
Mass increase %	22.4	3.60-47.3	17.8	8
Initial area (cm^2)	9.47	8.13-10.4	0.911	10
Maximum area (cm^2)	10.2	8.35-11.5	0.885	8
Surface area increase %	9.01	0.00-34.7	13.4	8
Maximum length (mm)	23.8	22.4-25.6	1.04	8
Length increase %	1.19	-2.14-8.49	3.94	8
Maximum breadth (mm)	13.7	11.4-15.4	1.14	8
Breadth increase %	7.21	-0.15-24.2	8.05	8
Relative clutch mass	36.7	33.5-38.5	2.03	5
Incubation at 25 °C (days)	85	85	NA	1
Incubation at 30 °C (days)	55.5	55-56	NA	2
Hatchling SVL (mm)	34.9	30-37	3.26	4
Hatchling tail (mm)	14.7	12-16	1.89	4
Hatchling mass (g)	1.67	1.02-2.10	0.468	4
Hatchling mass as % egg mass	74.8	56-84	13	4
Mean clutch size	2.0			

*Data include published measurements of one clutch of eggs (Delean and Harvey, 1984), a typographic error of 23 mm egg breadth has been corrected to 13 mm (confirmed by back-calculation from mass).

Nephrurus laevissimus

Four clutches of two eggs of *N. laevissimus* eggs were analysed (Table 5.8). *Nephrurus laevissimus* has the lowest relative egg size of all *Nephrurus* geckos.

Table 5.8 Egg data for *N. laevissimus*. STD = standard deviation, N = number of measurements.

CHARACTER	MEAN	RANGE	STD	N
Length (mm)	19.5	18-22	1.31	8
Breadth (mm)	10.1	9-11	.835	8
Length/breadth ratio	1.93	1.73-2.20	.168	8
Length + breadth as % SVL	31.2	28.4-33.7	1.86	8
Volume (mL)	1.056	0.763-1.27	.200	8
Mass (g)*	1.164	0.84-1.40	.221	8
Mean clutch size	2.0			

*Calculated using a mean density of 1.102g mL^{-1} found in *N. levis* and *N. asper* eggs. Measurements of eight eggs supplied by Mr Neil Sonnemann (personal communication, no eggs hatched as part of this study).

Nephrurus sheai

No live specimens or eggs of *N. sheai* were observed during this study. The only published egg data are from Gow (1979) (as *N. asper*). Eggs from a single clutch of *N. sheai* eggs measured 30 mm x 16.5 mm and 29 mm x 15.5 mm (Gow, 1979). The eggs were laid in early summer (wet season) at Katherine, NT, but both eggs failed to hatch when incubated in damp peat moss. No egg mass data were provided. Oviposition was not observed but a photograph of the eggs indicates they were well covered in substrate material. The mean length to breadth ratio is 1.84 and the mean egg mass is calculated to be approximately 4.7 g. The gravid female measured 134 mm SVL and the mean egg length plus breadth was 34.0 % of SVL (compared to 26.2 % in *U. milii* and 35.3 % in *N. amyae*).

Nephrurus levis

Nineteen *N. levis* two-egg clutches and three one egg clutches were analysed. The RCM and egg size index are both intermediate among *Nephrurus* species. The increase in mass and surface are the largest among *Nephrurus* species (Table 5.9). No published egg data are available for *N. levis*. Incubation duration is not significantly different at –100 kPa and –450 kPa ($P>0.05$). No Q_{10} was calculated because egg retention produced a wide spread in the duration of incubation. The mean conductance of *N. levis* eggshells was 91.4 ± 20.2 $mg.day^{-1}.torr^{-1}$ (N = 5).

Table 5.9 Egg Data for *N. levis*. STD = standard deviation, N = number of measurements.

CHARACTER	MEAN	RANGE	STD	N
Initial length (mm)	24.5	20.8-27.4	1.44	41
Initial breadth (mm)	13.8	11.5-16.1	1.02	41
Length/breadth ratio	1.78	1.54-2.00	0.100	41
Length +breadth as % SVL	39.1	33.8-43.4	2.28	41
Volume (mL)	2.46	1.50-3.37	0.464	41
Initial wet mass (g)	2.48	1.66-3.31	0.399	46
Calculated density (g/mL)	1.03	0.858-1.13	0.065	41
Maximum mass (g)	2.98	0.689-4.71	0.893	41
Mass increase % (hatched eggs)	26.5	-5.4-103	26.5	20
Initial area (cm^2)	10.7	7.84-13.0	1.29	37
Maximum area (cm^2)	11.4	8.40-16.3	1.92	18
Surface area increase %	9.45	-6.75-39.7	11.0	18
Maximum length (mm)	24.4	20.4-30.2	2.14	18
Length increase %	2.19	-2.01-21.7	5.68	18
Maximum breadth (mm)	14.8	12.9-17.6	1.42	18
Breadth increase %	5.90	0.000-17.8	5.36	18
Relative clutch mass	29.6	24.8-37.2	4.51	7
Incubation 25°C, -100kPa (days)	94.8	69-111	17.6	5
Incubation 25°C, -450kPa (days)	97.7	86-112	13.2	3
Incubation 30°C, -100kPa (days)	48.7	19-64	25.7	3
Incubation 30°C, -450kPa (days)	50.0	22-62	14.8	6
Hatchling mass (g)	1.94	0.876-2.95	0.413	20
Hatchling mass as % egg mass	76.5	34.3-96.3	16.7	18
Mean clutch size	1.78			

Mean measured density of *N. levis* (N = 2) and *N. asper* (N = 3) eggs were similar at 1.102 ± 0.058$g.mL^{-1}$.

Nephrurus stellatus

Four two-egg clutches of *N. stellatus* eggs show the largest egg size index (40.6 %) of all *Nephrurus* and *Underwoodisaurus* species, with a correspondingly large initial mass (Table 5.10). The index is significantly larger than for *U. milii* (32.6 %) ($t_{(1)87} = 5.894$, $P = < 0.0001$).

Table 5.10 Egg data for *N. stellatus**. STD = standard deviation, N = number of measurements.

CHARACTER	AVERAGE	RANGE	STD	N
Initial length (mm)	22.4	21.8-24.3	0.656	8
Initial breadth (mm)	12.5	11.5-13.7	0.866	8
Length/breadth ratio	1.82	1.72-1.94	0.083	8
Length + breadth as % SVL	40.6	38.7-43.4	1.72	6
Volume (mL)	1.85	1.51-2.32	0.307	6
Initial wet mass (g)	1.99	1.96-2.02	0.042	6
Initial area (cm^2)	8.82	7.88-10.16	0.854	6
Mean clutch size	2			

* Limited egg data of eight eggs from Mr Robert Porter and Mr Peter Page (personal communications, no *N. stellatus* eggs were hatched as part of this study).

5.3.8 Eggs of *Underwoodisaurus* species

Underwoodisaurus milii

Thirty nine *U. milii* clutches of two eggs and five clutches of just one egg were analysed (Table 5.11). The RCM is the smallest and the egg size index second smallest among *Nephrurus* and *Underwoodisaurus* species. The increase in mass and surface area are the greatest among *Nephrurus* and *Underwoodisaurus* species. Two eggs incubated at 35°C failed to hatch. The Q_{10} for incubation duration of *U. milii* eggs at 25 °C and 30 °C was 2.27. The mean conductance of *U. milii* eggshells was 50.9 ± 10.7 $mg.day^{-1}.torr^{-1}$ (N = 5).

Table 5.11 Egg data for *U. milii*. STD = standard deviation, N = number of measurements.

CHARACTER	MEAN	RANGE	STD	N
Initial length (mm)	23.2	20.1-28.2	1.71	83
Initial breadth (mm)	12.2	8.75-15.1	0.923	83
Length/breadth ratio	1.89	1.65-2.16	0.102	83
Length + breadth as % SVL	32.6	29.4-37.1	1.99	83
Volume (mL)	1.91	0.816-2.97	0.377	83
$3\sqrt{}$Volume	1.22	0.934-1.44	0.084	83
Initial wet mass (g)	1.91	0.775-3.02	0.396	83
Density (g/mL)	1.06	0.875-1.30	0.070	73
Maximum mass (g)	2.65	1.44-3.94	0.598	53
Mass increase %	34.4	-4.67-137	27.2	52
Mass increase % hatched eggs	39.2	12.9-95.9	20.3	27
Mass increase % infertile eggs	26.8	-4.7-1367	33.8	20
Initial area (cm^2)	8.96	5.59-11.8	1.21	78
Maximum area (cm^2)	10.3	7.62-12.0	1.09	37
Surface area increase %	18.0	3.28-43.6	10.1	37
Maximum length (mm)	23.4	20.2-26.0	1.31	40
Length increase %	0.631	-6.10-7.04	2.71	40
Maximum breadth (mm)	14.0	11.9-15.6	1.04	37
Breadth increase %	14.7	1.61-34.2	7.34	37
Relative clutch mass	24.7	14.734.9	6.04	15
Incubation 25°C, -100kPa (days)	83.3	79-87	4.35	4
Incubation 25°C, -450kPa (days)	74.4	62-83	7.44	8
Incubation 30°C, -100kPa (days)	53	50-56	3.00	3
Incubation 30°C, -450kPa (days)	51.5	50-53	2.12	2
Hatchling mass (g)	1.60	0.973-2.05	0.294	33
Hatchling mass as % egg mass	83.6	54.0-106	11.0	30
Mean clutch size	1.82			

Underwoodisaurus sphyrurus

One clutch of two *U. sphyrurus* eggs was analyzed (Table 5.12). The relative clutch mass and relative egg size are both very large.

Table 5.12 Egg Data for *U. sphyrurus**. STD = standard deviation, N = number of measurements.

CHARACTER	MEAN	RANGE	STD	N
Length (mm)	21.7	21.3-22.7	NA	2
Breadth (mm)	10.9	10.7-1.1	NA	2
Initial mass (g)	1.53	1.51-1.55	NA	2
Length/breadth ratio	1.99	1.92-2.07	NA	2
Length + breadth as % SVL	38.4	37.6-39.8	NA	2
Relative clutch mass	30.8	NA	NA	1
Mass increase (%)	15.7	0.2-31.1	NA	2
Hatchling SVL (mm)	35	NA	NA	1
Hatchling mass (g)	1.31g	NA	NA	1

* The measurements for one hatchling have been provided by Mr Robert Porter (personal communication). NA = not applicable.

5.4 Discussion

5.4.1 Nest Site Selection and Egg-laying

Nephrurus and *Underwoodisaurus* species are thought to deposit their eggs in damp sand or similar substrate (Gow, 1979; Wagner and Lazik, 1996). The female gecko may influence the environment to which her eggs are exposed through the seasonal timing of egg laying, nest site selection and nest construction (Ackerman, et al. 1985; Ackerman, 1991). In captivity, nesting burrows are invariably located adjacent to a protective or vertical structure. Nest site selection is critical to survival of flexible-shelled eggs (Thompson, 1990; Anderson, 1993), but factors that guide maternal behaviour in site selection are unknown. The digging of what appear to be 'test' burrows suggest that the gecko is able to evaluate some characteristics of the substrate. The fact that females are quite prepared to

dig vigorously in dry, free-flowing surface sand suggests that a firm or moist substrate surface is not a prerequisite for nest site selection.

The presence of vegetation in the habitat (Appendix 5) provides shade, thus reducing temperature and humidity fluctuation in the soil surface; surface rocks may also reduce the rate of evaporation from the soil. Vegetation and rocks will probably both help reduce wind erosion thus providing a more stable environment for egg incubation. The behaviour in captivity of *N. asper, N. levis* and *U. milii* of burying eggs adjacent to or beneath a large heat sink or insulating layer could reduce the extremes of temperature to which the eggs might be exposed in the wild. Nesting burrows are deep (usually at the bottom of the vivarium or nesting box - up to 200 mm deep). In the wild, nesting burrows are therefore probably even deeper, where the diel temperature fluctuations are much less (Packard, G. C. and Packard, M. J., 1988; Palmer-Allen, et al., 1991), the water potential is more stable (Packard, et al., 1992), and the eggs are less likely to be damaged by predators or flash flooding.

Some gecko species have asynchronous laying of the two eggs of a clutch, possibly at separate locations or a few days apart (*e.g.,* Szczerbak and Golubev, 1996; Rogner, 1997). *Aeluroscalabotes felinus* has asynchronous development of the two oviductal eggs of a clutch (Werner, 1972). The few cases of unusual egg-laying variations found in this study may have been due to captive conditions, and little significance can be attached to them. The finding of up to 20.8 % of clutches in *Nephrurus* and *Underwoodisaurus* species consisting of only one egg may also not represent the wild situation; in captivity the husbandry regime and a preponderance of young females may have contributed to an increased proportion of one egg clutches.

Immediately after egg laying, the female (among both diplodactyline and gekkonine species) commonly rolls the eggs around using the hind legs, feet and tail (Greenberg, 1943; Greer, 1967; Seufer, 1991; Girard, 1993; McKeown, 1993; Henkel and Schmidt, 1995). In many gecko species, egg rolling involves the application of substrate material to the shell, but in some gekkonine species, although egg rolling still occurs, no substrate material adheres to the eggshells (Henkel, 1992; Rogner, 1997).

Many diplodactyline (and gekkonine) species (including *Nephrurus* and *Underwoodisaurus*) give their eggs a thick coat of sand during egg rolling (Henkel and Schmidt, 1995), suggesting an adaptive advantage may be involved. Egg rolling in geckos may serve a different function to avian egg turning (Poulsen, 1953; Deeming, 1991); it may shape the egg or make it more spherical (Miller, 1984) or may improve camouflage (Henkel and Schmidt, 1995). Eggshells covered in sand may be less palatable to some predators. The fact that the sand layer is often thick and continuous (unlike that found *e.g.*,in *Cyrtodactylus louisiadensis* eggs laid in the sand) suggests that the mucoid surface layer is different from that found in some gekkonine eggs and that it is not merely a by-product of rolling the wet egg. In *Nephrurus* and *Underwoodisaurus* species eggs, camouflage is unlikely to be of value to buried eggs. Also, the shape of the tough shell membrane is unlikely to be altered by rolling, and therefore a desiccation limiting function is thought more likely. Repeated manipulation of eggs in a laboratory situation always disrupts some of the compact layer of sand, which may explain why desiccation rates of thickly covered eggs were not significantly different from those with less sand attached ($P>0.05$). Also, the fact that removed sand grains leave craters in the egg surface implies that the wet surface layer has been pushed aside into the crevices between sand grains, increasing the effective thickness of the shell. Even limited inhibition of water loss would be important in improving arid region egg survival, especially if the egg had already acquired an increased water reservoir.

5.4.2 Clutch Size

A clutch of eggs in geckos is normally either one or two eggs; if two, they are usually laid close together at one time. The invariance of clutch size in geckos (Vitt, 1986; Shine and Greer, 1991) indicates that geckos are limited in the control of their fecundity. Geckos may be able to vary the number of clutches per season (depending on temperatures and nutrition) and for those species with increased longevity, a greater number of clutches may be laid in one life cycle.

There is no convincing evidence that any gecko species has a normal clutch size greater than two eggs. Clutches of three or more eggs have been occasionally recorded in diplodactyline, gekkonine and eublepharine geckos (over thirty such events are listed in

Appendix 7, Table A7.2). *Uroplatus henkeli* and *Geckoella yakhuna* have been recorded as laying a clutch of four eggs (Henkel and Schmidt, 1995; Deraniyagala, 1953), as do some eublepharine geckos (Goin, et al, 1978; Zug, 1991). Clutch sizes of up to six eggs are reported in *Hemidactylus triedrus lankae* and in *Gymnodactylus frenatus* (Deraniyagala, 1953). A claim of '5 to 6 eggs' for normal clutch size in *Hemidactylus flaviviridis* (Kelaart, 1852) is countered by a claim of 1-2 eggs (Arnold, 1984). A report of 6-12 eggs being laid in rows by *Phelsuma guentheri* (Pike, 1873), and probably all reports of more than four eggs in a clutch, probably represent either multiple clutching by one female or communal laying by more than one female.

Multiple clutching was distinguished from staggered laying of single eggs, in this study, by regular observation of captive females during the breeding season. The fact that gravid specimens of several *Nephrurus* species taken from the wild have been found in spring, and throughout summer (How et al., 1991) supports the possibility that multiple clutching may also occur in the wild. The moderate sized diplodactyline, *Strophurus spinigerus*, has been observed carrying '3-4' eggs with different sized eggs in an oviduct, possibly implying staggered egg-laying (Dell and Chapman, 1977). However, eggs probably cannot 'grow' in the oviduct, the shell is laid down at full size in the uterus soon after ovulation (Iverson and Ewert, 1991). Thus, the presence of eggs of different sizes probably indicates that the smaller eggs were defective or possibly representing the early pre-shelling stage of egg formation of the following clutch of eggs.

Many gecko species have a fixed clutch size of one egg (Appendix 7, Table A7.2). The majority are tropical *e.g.*, the small to very small sphaerodactyline geckos (Schmidt and Inger, 1957; Smith, H. M., et al, 1977; Miller, 1984; Henkel and Schmidt, 1995). The sphaerodactyline geckos have a long reproductive season to increase fecundity e.g., *Gonatodes humeralis* lays up to 12 clutches per year (Henkel and Schmidt, 1995). The reason why some diplodactyline geckos sometimes lay only one egg per clutch is not known, but the fact that the eggs of one-egg clutches are larger than eggs from two-egg clutches suggests that the offspring may also be larger and thus convey a selective advantage.

5.4.3 Egg Size

Egg size and egg shape are closely related, and surface area-volume relationships are of great biological significance *e.g.,* in diffusion of gases (Deeming and Thompson, 1991; Solomon et al., 2002), evaporation (Ar, 1991; Hall, 2001) and the transfer of thermal energy (Ackerman, et al., 1985; Rhoades and Pflanzer, 1996). If egg size and shape are the result of natural selection, then variations in size and shape must reflect adaptations to environmental conditions (Hoyt, 1976).

One major characteristic of the data sets (including *N. levis* and *U. milii*) is that there is a large intraspecific variance in the egg dimensions (Appendix 6) in apparently normal eggs. Such variation is, however, normal in the Gekkoninae (Cagle, 1946; Anderson, 1964; Bustard, 1968a; Chou, 1979; Cogger, et al., 1983b; Gibbons and Zug, 1987; Werner, 1989), the Eublepharinae (Werner, 1972) and the Diplodactylinae (Bustard, 1967c).

Several factors may select for increased egg size. For example, if the hatchlings are at greater risk than the eggs, then natural selection will favour the production of larger eggs and hatchlings (Shine, 1978a, 1985b). Similarly, if smaller eggs are at greater risk in an arid environment, then natural selection will favour the production of larger eggs. Desiccation is probably a major selective stressor in oviparous arid-adapted species laying flexible-shelled eggs, especially in abnormally dry seasons. The diplodactyline and eublepharine geckos produce relatively larger eggs than geckos laying rigid-shelled eggs. The difference suggests that there is something fundamentally different in reproductive strategy between the groups. Most geckos in both groups are nocturnal arthropod feeders, and many are broadly sympatric. Also, many species in each group are terrestrial while others are arboreal, suggesting that some other difference may be largely responsible for the relative egg-size difference. The most obvious difference is the dichotomy in eggshell type. The major functional difference between the two egg types is the difference in conductance. Diplodactyline eggs can lose water at a rate that is lethal within hours, whereas gekkonine eggs do not. Diplodactyline eggs are also relatively larger than the gekkonine eggs. If a diplodactyline egg with a larger reservoir of water is more likely to survive in an arid environment, then that characteristic will be selected for and larger relative egg size in arid-adapted diplodactylines, compared to gekkonines or mesic diplodactylines, will result. The

relatively larger eggs among Australian diplodactylines compared to Australian gekkonines on its own may not be significant, but support of the relation of egg size to aridity is provided by the comparison of *Nephrurus* and *Underwoodisaurus* eggs. Relative egg size in (pooled) *Nephrurus* species is significantly greater than in *Underwoodisaurus* species ($t_{(2)170} = 3.967$, P = <0.0001). Additionally, the relative egg size of arid-adapted diplodactylines is significantly larger than non-arid-adapted diplodactylines ($t_{2(28)} = 2.917$, P = 0.003), supporting the hypothesis that aridity (superimposed on the phylogenetic constraint of large eggs) has an influence on egg size.

Larger lizard eggs usually mean larger hatchlings (Greer, 1989), and larger offspring mean better quality offspring that are more likely to survive to maturity (Sinervo, 1994). Thus, selection may be for larger hatchlings rather than for larger eggs, and it is probably impossible to separate the two selection pressures. The fact that many small skinks and dragons (that lay flexible-shelled eggs) flourish in arid Australia (Cogger, 2000) suggests that small egg size *per se* is not necessarily a problem.

Egg size among gecko species is strongly correlated with maternal size (How, Dell and Wellington, 1986; Vitt, 1986), but the sizes of the rigid-shelled eggs relative to maximum maternal snout-vent lengths are significantly smaller than the relative sizes of the flexible-shelled eggs (Figure 5.6). At least 20 gekkonine species (Minton, 1966; Greer, 1989; Werner, 1989; Seufer, 1991; Leptien, 1992; Arnold and Gardner, 1994; Henkel and Schmidt, 1995; Szczerbak and Golubev 1996; Vitt and Zani, 1997; Donnellan, Aplin and Dempsey, 2000; Cronin, 2001; Appendix 7, Table A7.2) and all sphaerodactyline species (Miller, 1984; Schwartz and Henderson, 1991) have only single egg clutches, and therefore might be expected to produce relatively larger eggs. However, the difference in relative egg sizes in the diplodactyline and gekkonine groups remains. The clutch size is unconfirmed for a majority of gekkonine species, also a number of species produce either one or two eggs (Minton, 1966; Seufer, 1991; Szczerbak and Golubev 1996; Doughty and Thompson, 1998; Donnellan, Aplin and Dempsey, 2000; Appendix 7, Table A7.2). *Nephrurus* and *Underwoodisaurus* species also sometimes produce only one egg per clutch.

If there is a selective advantage in producing larger eggs among *Nephrurus* geckos, then it can be predicted that the eggs produced would be close to the maximum size limit and that

the limit might be set by either resource availability or female body size (Smith and Fretwell, 1974; Ferguson, et al, 1982; Shine, 1989a; Shine, 1991; Frankenberg and Werner, 1992). The larger eggs found in *Nephrurus/ Underwoodisaurus* one-egg clutches than in two egg clutches suggests possible resource limitation when producing two eggs, and also suggests that the bony pelvis may not be the major limiting factor for increased egg size. An alternative explanation is that the intra-abdominal changes generated by the rapid formation of two large eggs could produce a mutual inhibition in the oviducts on maximum size of the eggs, even without resource limitation or pelvic outlet limitation (Iverson and Ewert, 1991).

The slopes of the linear regressions of the relative egg sizes in diplodactyline and Australian gekkonines are significantly different (Figure 5.7). If the very large *Rhacodactylus* species that produce highly calcified eggs (personal observation), are excluded from the analysis the difference in slope is further increased. This evidence with a reduced conductance supports the hypothesis of reduced selection pressure towards increased gekkonine egg size. The increased regression slope of relative egg size when *Rhacodactylus* is excluded from the comparison (Figure 5.8) suggests that there is greater selection pressure on Australian diplodactyline species (including *Nephrurus* and *Underwoodisaurus*) to produce larger eggs than in the gekkonine species. Conversely, it can be argued that the data support selection pressure on the gekkonine species to produce smaller, and therefore energetically less costly, eggs.

The limited data for *N. laevissimus* eggs (Table 5.8) indicate relatively very small eggs, which does not fit well with the hypothesis that arid species produce relatively larger eggs, the mean relative egg size of *N. laevissimus* is intermediate between *U. milii* and *U. sphyrurus*. Further live egg data are needed to clarify this possible difference from other *Nephrurus* species eggs.

Nephrurus stellatus is the smallest species in the genus (as defined by maximum female SVL of 86 mm, N = 13) and (Table 5.10) produces the smallest eggs (mean 1.99 g, N = 6) of the genus (with the possible exception of *N. laevissimus* eggs). Nevertheless, it produces relatively large eggs (the mean egg size index is 40.6 %) compared to a range of 35.1-54.0 % for the genus.

The limited RCM data available (Appendix 6, Table A6.9) also supports the hypothesis that diplodactylines produce relatively larger eggs than gekkonines (P = < 0.001. The four *Nephrurus* mean RCM values obtained in this study are consistently high (*N. deleani* 36.7 %, *N. levis* 29.6 %, *N. asper* 29.3 %, *N. amyae* 28.0 %) and significantly greater than for *U. milii* (24.7 %) (Table 5.11) (a single value of 30.8 % was obtained for the smaller *U. sphyrurus*) (Table A6.9) ($t_{(1)34}$ = 2.9505, P = 0.0029). These values compare with a range of 16-39 % in 18 Australian diplodactyline species (Appendix 6, Table A6.9). Mesic diplodactylines (*Carphodactylus laevis, Diplodactylus taenicauda, Oedura castelnaui* and *Phyllurus platurus*) have a significantly smaller RCM than *Nephrurus* species ($t_{(1)8}$ = 2.958, P = 0.0091). The RCM values obtained for *Nephrurus* and mesic diplodactyline species are all supportive of the hypothesis that egg size is increased in arid species.

5.4.4 Egg Shape

Reptilian eggs differ from avian eggs in only rarely being tapered (Preston, 1968; Smart, 1991; Iverson and Ewert, 1991). Egg shape among reptiles is more variable than among birds (Iverson and Ewert, 1991), but an analysis of egg shape in birds concluded that there was no effect on embryonic respiratory physiology (Smart, 1991).

Many reptile eggs are flexible-shelled and may increase or decrease in mass as water is absorbed or lost (Muth, 1981). In flexible-shelled eggs the effective shell thickness decreases due to stretching and possibly due to reabsorption of calcium from the shell as embryogenesis proceeds (Simkiss, 1962, 1991; Packard, M. J., 1990), thus enhancing gas and water exchange rates.

Elongated eggs allow an increased egg mass, producing larger hatchlings without a corresponding increase in maternal internal pelvic diameter (Hailey and Loumbourdis, 1988). Egg elongation also increases the surface area to mass ratio and probably decreases the strength/shell thickness ratio. Reptilian eggs exhibit a wide range of length-breadth ratios, from spherical in some geckos (*e.g.,* Loveridge, 1947, 1961; Werner, 1989) to highly elongated in some amphisbaenians (*e.g.,* the *Blanus cinereus* egg is approximately 30.5 mm long and 5.6 mm wide (Salvador, 1985) and the *Chirindia ewerbecki* egg measures 31 mm

long by 2 mm wide (Loveridge, 1942a). The length: breadth ratio for Australian gekkonine eggs ranges from 1.1 to 1.7 and from 1.5 to 2.0 for diplodactyline eggs (Greer, 1989).

An elongated flexible-shelled egg allows the egg to absorb more water during embryonic development without exceeding the rupture pressure. Conversely, a spherical or near spherical egg would be better able to withstand the forces generated by increased or decreased internal pressures because the forces are evenly distributed on a spherical surface.

Large intraspecific variations of the LBR have been published *e.g., Teratolepis fasciata*, ranges from 1.00 to 1.31 (N = 14) (Anderson 1964). Wherever multiple numbers of gecko egg measurements have been made (*e.g.,* Cagle, 1946; Anderson, 1964; Bustard, 1967c, 1968a; Chou, 1979; Cogger et al., 1983b; Gibbons and Zug, 1987; Werner, 1989; Douglas, 1990; Henkel and Schmidt, 1995; Szczerbak and Golubev, 1996), they all indicate a large intraspecific variation. Thus, variation in the LBR in *Nephrurus* and *Underwoodisaurus* species eggs can be expected.

The mean value for the LBR varies little among *Nephrurus* species, (range 1.73 ± 0.07 in *N. asper* to 1.84 ± 0.12 in *N. deleani*). The LBR of 2.30 for *N. amyae* (as *N. asper*) (Bedford and Christian, 1993) is for eggs that failed to hatch and the LBR value may be abnormal. The measurements show that the largest *Nephrurus* eggs are more elongate when laid, suggesting that an upper limit of egg size determined by the maternal pelvic diameter (Iverson and Ewert, 1991) may be partially overcome by the elongation of the eggs.

An argument against the aridity-enlargement hypothesis relates to the distribution of the closely related *N. amyae, N. asper* and *N. sheai* group. The distribution of the species *N. amyae* is centred near Alice Springs, Northern Territory where the mean annual rainfall is approximately 270 mm per year (Chapter 2). In contrast, some populations of *N. sheai* and *N. asper* occur in areas where the mean annual rainfall is monsoonal at 1500 - 2100 mm per year, but these species still produce relatively large eggs.

The presence of isolated northern populations of *U. milii* (Chapter 2), where they survive in deep, cool crevices (Cronin, 2001), (although broadly sympatric, none of the localities for *U. milii* in the Northern Territory matched the localities for *N. amyae*) suggests that this

species was more widely distributed in the past. The desertification of central Australia since the late Pleistocene (Keast, 1959) may have eliminated most of the central *U. milii* populations, but allowed *Nephrurus* species to radiate across central and northern Australia.

The large intraspecific variation in bicone values means that a species-specific value for accurate calculation of egg volume or density was not appropriate. Also, because diplodactyline eggs change shape during embryonic development, the bicone values also change, contributing to intraspecific variation in egg shape. The reason that the calculated volumes (using the formula for a prolate ellipsoidal shape) and densities were less than the measured values is that all bicone measurements of viable eggs were positive. This probably also explains why some published values of relative density for gekkonine species eggs were calculated as being less than one (Douglas, 1990). Additional uncertainty is introduced when using published egg sizes, as the time of measurement after oviposition in many cases is uncertain.

5.4.5 Structure and Ultrastructure of Gecko Eggshells

The eggshell plays a pivotal role in the embryonic development of oviparous reptile species, so analysis of eggshell structure-function relationships will help determine the limits of any adaptations to aridity. A thicker eggshell in *Nephrurus* compared to *Underwoodisaurus* species eggs was not confirmed. The rigidity of brittle-shelled eggs is due to the presence of an outer layer of calcium carbonate, usually in the form of calcite (Silyn-Roberts, and Sharp, 1985; King and Horner, 1993). The diplodactyline eggshell is similar to the generalised squamate eggshell, in which four layers are present, a thin outer calcareous cover (that varies between and within species), followed by a thin condensed surface layer (a slightly more dense outer region of the third layer), then the fibrous membrana testacea (the major part of the shell membrane), plus a thin, smooth inner boundary layer (Schleich and Kästle, 1988). Macroscopically, live *Nephrurus* and *Underwoodisaurus* eggs (like other diplodactyline eggs) appear smooth, matt, opaque white and of leathery flexibility. The inorganic layer of squamate eggshells is calcium carbonate (Schleich and Kästle, 1988; Packard, M. J. and DeMarco, 1991; King and Horner, 1993). There was no evidence in *Nephrurus* or *Underwoodisaurus* eggshells of elongated orthorhombic or twinned prismatic crystals, as is commonly found in aragonite (Roberts, et

al., 1990). The finding of a hard meniscus around attached sand grains (Figure 5.10) shows that the mucoid surface layer includes calcium carbonate and is still soft when the substrate material is applied. Bare patches, with little or no sand attached, may result from disturbed egg-laying or because of captive conditions. Complete drying and hardening of gekkonine eggshells takes about four hours (Cagle, 1946), allowing ample time for application of substrate material.

Eggshells must be strong enough to provide protection and also sufficiently permeable to allow adequate gas exchange for embryogenesis to proceed (Ackerman, 1977). Therefore knowledge of the physical characteristics of the eggshell is important in understanding the physiology and ecology of embryos during development (Werner, 1989). The major forces that are liable to impact the egg are:

1. peristaltic pressures as the egg passes through the oviduct and cloaca (Smart, 1991), 2. rolling of the egg by the parent gecko (Greer, 1967; Rogner, 1997),
3. substrate compression of burial process (Seymour and Ackerman, 1980; personal observation),
4. movements of the embryo, particularly as hatching approaches (Armstrong, 1979; personal observation), and
5. changes in shell tensile forces due to exchange of water with the environment (Packard, 1991).

6. Erosion of shell calcium by the developing embryo (Packard and DeMarco, 1991; Simkiss, 1991) may also weaken the shell.
7. Predator attacks (personal observation).

In addition, defective shell formation or microbial attack may alter shell characteristics.

Probably all reptilian eggshells contain some calcium carbonate (Packard, M. J. and DeMarco, 1991), but it is evident that the relatively thick outer layer found in gekkonine eggshells has a major effect on reproduction and egg physiology when compared to diplodactyline eggs. Occasionally, eggs are laid (both gekkonine and diplodactyline) with translucent patches, indicating local calcium carbonate deficiency (Figure 5.9), which does not necessarily impair embryonic development. The fact that the calcium carbonate is laid

down in patches rather than swirls suggests that the egg is not rotating (or rotating only slowly or intermittently) during the calcification process.

Macroscopically and microscopically *Nephrurus* and *Underwoodisaurus* eggshells appear similar to those of other flexible-shelled diplodactylines (Packard and Hirsch, 1986). The SEM appearance shows that the calcareous component of *Nephrurus* and *Underwoodisaurus* eggs consists of a crust of very fine calcite crystals, which are not highly organised or as thick as are found in rigid-shelled *Gehyra variegata* (this study) or other gekkonine eggshells (Packard and Hirsch, 1989). The SEM surface topography is slightly irregular, but is continuous apart from occasional small cracks and scattered small pores of irregular size and shape and low density. In avian eggs the conductance of the shell is proportional to the pore number (Ar and Rahn, 1985). However, because of low pore density and poorly organised calcite of *Nephrurus* and *Underwoodisaurus* eggshells, it is apparent that their conductance must be determined largely by the passage of gases directly across the entire and intact eggshell.

The transmission electron microscopic appearance of the outer shell junction with the membrana testacea (Figure 5.11) shows numerous spaces where diffusion could occur. The membrana testacea forms a dense mat of protein fibrils (Figure 5.12) intermingled with calcite crystals in the outer zone (the calcite is presumably laid down after the membrana testacea is completed). Fused fibrils associated with organic globules occur in some reptilian eggs (Schleich and Kästle, 1988). Although fibrils may appear stuck together, no fused fibrils were seen in *Nephrurus* or *Underwoodisaurus* eggshells. The inner boundary layer is smooth, very thin (< 1 μm) and without reticulations or microvilli (Figure 5.13) (Schleich and Kästle (1988). Little is known about the inner boundary layer in reptiles (Packard, M. J. and DeMarco, 1991), but it represents an important resistance to diffusion of gases in birds (Tranter, et al, 1983). The inner boundary layer is presumably the layer that prevents the fluid substance of the yolk from seeping through the fibrils of the membrana testacea.

The presence of calcium carbonate in the eggshell is demonstrated by the large amount of ash in *Nephrurus* and *Underwoodisaurus* eggshells (41.9 % ± 9.76, N = 10) and confirmed by EDS analysis of the shell (Figure 5.16). Although the SEM

shows a large amount of calcium in *Nephrurus* and *Underwoodisaurus* shells, the structure of pores is not highly organised as it is in avian eggshells (Tyler, 1969; Booth and Thompson, 1991), and the SEM appearance in the rigid-shelled eggs of *Gehyra variegata* is intermediate in terms of pore structure. The outer shell layer has much more calcium than the inner layer. The inner layer of the shell has little calcium but considerable sulphur, presumably representing the presence of the sulphur-containing amino acids (cysteine and methionine) in extracellular protein fibrils (Stryer, 1995; Palmer and Guillette, 1991). This structure also suggests that there are two separate sections of the oviduct that lay down the fibril and calcite layers sequentially.

No loops or 'V' orientated fibrils were seen in *Nephrurus* or *Underwoodisaurus* eggshells, indicating that rotation of the egg in the oviduct during shell formation is probably continuous in one direction and not oscillatory or reversed. The egg probably rotates relative to the oviductal shell gland in a sinusoidal fashion to ensure even coverage of the ends of the egg. The layered criss-cross orientation of shell fibrils indicates that the egg is rotated (relative to the oviduct) more-or-less alternately along two perpendicular axes during this component of shell formation (Figure 5.17, Schleich and Kästle, 1988). The resulting even distribution of the fibrils thus gives strength, allowing stretch during embryonic development but usually preventing defects or rupture of the growing egg.

Egg density increases non-linearly with the amount of calcium in the shell and, conversely, the presence of more lipids in an egg will reduce the mean egg density. The density of gekkonine eggs might be slightly greater than in carphodactyline eggs because of the relatively larger amounts of calcium carbonate in the shell (Packard, M. J. and DeMarco, 1991), but the increased sphericity of gekkonine eggs will reduce the surface area to mass ratio. I found no published density values for flexible shelled gecko eggs but the mean value of *Nephrurus* species egg density (1.04) is within the range (0.92-1.14) given for four species of rigid-shelled gecko eggs (Douglas, 1990), and at the lower limit of the range (1.045 - 1.109) given for 13 species of flexible-shelled turtle eggs (Iverson and Ewert, 1991).

5.4.6 Incubation, Temperature and Water Potential

The mass of rigid-shelled eggs of gekkonines generally remains virtually constant throughout incubation, because of the low water vapour conductance, lack of elasticity in the shell, lack of an air cell (as in avian eggs) (Dunson, 1982; Booth and Thompson, 1991; Deeming and Thompson, 1991; Iverson and Ewert, 1991; Paganelli, 1991), and presumably high surface tension. New laid gekkonine eggs (of *Christinus marmoratus*) have approximately 80 % water content (Thompson and Russell, 1999b), which is essentially the same as found in diplodactyline eggs in this study but more than that found in some other lizards (*e.g.*, 73 % in *Pogona barbata* (as *Amphibolurus barbatus*) (Packard, M. J., et al, 1985), 60.1 % in *Eumeces fasciatus* (Thompson and Stewart, 1997) and 75.9 % in *Menetia greyii* (Thompson and Russell, 1998)). Why some gecko eggs need to take up water while others do not is not known, but if the production of larger eggs were advantageous in increasing desiccation resistance, then the ability to absorb water after laying would also be a significant advantage. Providing such eggs were deposited in a moist environment, the water reserve against possible future dehydration stress would be increased. The capacity to increase water content after laying also means that the female is able to lay eggs that are effectively larger than what the pelvic canal would otherwise allow. Also, the evidence suggests that water uptake is necessary for *Nephrurus* and *Underwoodisaurus* embryonic development, because eggs die if a positive water balance is not maintained.

There was no significant difference in the hatching rates of *N. levis* (48.8%) and *U. milii* (48.5%) eggs incubated under all four experimental conditions, suggesting that the conditions are all within the physiological limits of incubation. The highest hatching rate (86 %) was significantly higher among undisturbed eggs incubated at room temperatures, ($P = 0.03$) suggesting that the experimental manipulations may have had some detrimental effect on embryonic development.

All viable *Nephrurus* and *Underwoodisaurus* eggs increased in mass during incubation, and most increased continuously throughout incubation at a rate proportional to the increasing surface area, as in some other lizard species (*e.g., Sceloporus*, (Tracy, 1980) and *Anolis*, Andrews and Sexton, 1981), but unlike others (*e.g., Dipsosaurus*, (Muth, 1981) and *Pogona* (as *Amphibolurus*), (Packard, M. J. et al., 1985)) where an initial increase is

followed by a decrease in mass. Incubation substrate water potential affects hatchling mass and various metabolic processes in some reptiles (Deeming and Ferguson, 1991; Packard, 1991). In this study there was no significant difference in the relative mass of either *U. milii* or *N. levis* hatchlings (compared to initial egg mass) incubated at -100kPa and at -450kPa (P = >0.05), possibly because both regimes were within normal limits. The weak but positive correlation between initial egg mass and hatchling mass suggests that neither genetic nor environmental factors are dominating the determination of hatchling size.

The reason for the significantly greater rate of water uptake by *U. milii* eggs compared to *N. levis* eggs is not certain., The uptake of less water in larger eggs in the same water potential substrate implies that the conductance of *Nephrurus* eggshells is less than in *U. milii* eggshells. The measured eggshell conductances (and shell thicknesses) were not significantly different in *N. levis* compared to *U. milii* (P = >0.05). The mean surface area specific conductance values for *N. levis* and *U. milii* eggs (64.8 and 43.1 mg H_20. day^{-1}. kPa^{-1}. cm^{-2} respectively) were below the range for 21 (mainly non-arid) lizard species laying flexible-shelled eggs (Ackerman et al., 1985; Deeming and Thompson, 1991) (range 141 – 318 mg H_2O. day^{-1}. kPa^{-1}. cm^{-2}), which implies increased resistance to aridity in both *Nephrurus* and *Underwoodisaurus* species. These results are above the values for rigid-shelled gecko eggs 0.1 – 0.3 mg H_2O. day^{-1}. kPa^{-1}. cm^{-2} (Dunson and Bramham, 1981; Dunson, 1982).

That two fertile *Nephrurus* eggs failed to increase in surface area during incubation (increased breadth but decreased length), also suggests that *Nephrurus* eggshells are less compliant than *Underwoodisaurus* eggshells. The reduced compliance of *Nephrurus* must be due to increased strength of the shell membrane, as the calcium carbonate is not sufficiently well organised (as shown by electron microscopy) to contribute to the tensile strength of the shell. The reduced water uptake by *Nephrurus* eggshells also demonstrates that, although an increase in conductance is usual and advantageous, it is not essential for embryonic development.

Desiccation of flexible-shelled eggs during incubation at low (drier) water potentials may be detrimental or fatal to the developing embryo (Tracy, 1980; Andrews and Sexton, 1981; Muth, 1981; Ackerman, 1981; Packard, G. C., 1991). In contrast, low water potentials are

not lethal to rigid-shelled gecko eggs, although high substrate water potentials may reduce their hatching rates (Bustard, 1968c; Greer, 1989). The rate of moisture loss from rigid - shelled eggs is extremely low: zero in the gekkonine *Hemidactylus frenatus* (Schwaner, 1980) and <0.5 % of egg mass in *Christinus marmoratus* at -150 kPa (Thompson and Russell, 1999b). The rapid initial rate of weight loss upon exposure to a dry atmosphere by flexible-shelled eggs is followed by a long period of almost constant weight loss, suggesting that the initial moisture loss is probably due to evaporation from the surface and microscopic interstices of the shell. The eggshell conductance values obtained are low compared to other similar sized flexible-shelled eggs, suggesting that *Nephrurus* eggs may be better adapted for arid conditions.

As water is absorbed by the flexible-shelled eggs of *Nephrurus* and *Underwoodisaurus*, the hydrostatic pressure inside the egg will increase and the osmolality will fall, which will inhibit the uptake of more water, at least until the shell membrane has had time to stretch. Stretching of the eggshell and loosening of attached sand will increase conductance, thus facilitating water uptake. Counteracting the effects of swelling may be the compacting effect of the enlarging egg on the surrounding substrate and adjacent egg. Increase in egg mass is slow and matches the progress of embryonic development. The increasing conductance of the eggshell during incubation means that the rate of water uptake or loss could also increase.

To survive, *Nephrurus* and *Underwoodisaurus* eggs must be buried at a depth where the water potential is adequate to maintain positive water balance (probably wetter than -1500 kPa, Muth, 1981). The significantly greater increase in mass of *U. milii* eggs incubated at -100 kPa compared to eggs incubated at -450 kPa, shows that substrate water potential significantly affects water uptake, even between two relatively moist conditions. It also shows that embryos are capable of surviving a range of substrate water potentials (Chapter 6).

The metabolism of all reptile eggs can be significantly affected by available water during incubation (Packard, G. C., 1991). However, the mean incubation periods for *N. levis* eggs at the two different water potentials (-100 kPa and –450 kPa) were not significantly different at either 25 °C or at 30 °C incubation temperatures, indicating that small

variations in water potential of the incubation medium do not have a significant effect on duration of embryonic development. It is possible, however, that in this study egg retention has confounded the results by increasing the variance of incubation duration.

Most oviparous squamates retain the eggs for approximately half the embryonic development period (Shine, 1983). *Nephrurus* (species unspecified) eggs take 64-72 days to hatch at 28 °C, and as little as 45 days at 32 °C (Wagner and Lazik, 1996). No data are available for embryonic stage at laying for any Australian diplodactyline gecko. However, wide variations for incubation time for Australian gecko species have been found (Greer, 1989), suggesting there may be intraspecific and interspecific variation in embryonic stage at laying. Australian diplodactyline geckos have incubation periods of 43-88 days for seven species at 30°C (Greer, 1989). In *Nephrurus* and *Underwoodisaurus* the range is intermediate at 48-56 days (Tables, 5.4, 5.6, 5.8, 5.10). The low to intermediate incubation period found in this study is surprising considering the large size of the hatchlings, possibly indicating a high standard metabolic rate (Dial and Grismer, 1992).

The minimum incubation period in Australian geckos of 43 days at 30 °C for *Diplodactylus elderi* (Bustard, 1965a), suggests that there is no significant selection pressure towards placental development or viviparity among Australian diplodactyline species including *Nephrurus* and *Underwoodisaurus*.

In contrast to *N. levis*, the mean incubation for *U. milii* eggs at 25 °C was significantly increased in the wetter substrate. The significance of this difference is not known.

Even though there is considerable spread in the duration of incubation in *N. levis* at a given temperature and water potential, two eggs (a single clutch) showed a shorter incubation (19 days compared to a mean of 50 days for nine eggs incubated at 30 °C). This suggests some degree of egg retention, which may have been a result of factors such as low temperatures or dry substrate inhibiting the female from laying (Stamps, 1983) (low temperatures did not occur at this time, but vivarium substrate water potentials were not recorded). Reduced incubation period also implies continued embryonic development during egg retention. Egg retention has also been observed in an unspecified *Nephrurus* species (Wagner and Lazik, 1996), in *N. deleani* (Harvey, 1983) and in *U. milii* (personal observation). Egg retention

may be a selective advantage to an arid lizard where frequent, severe and prolonged droughts could compromise embryonic development. Both eggs of a clutch hatch almost simultaneously if incubated at the same temperature; however, the large standard deviation for incubation duration indicates some flexibility in the control of gestation period (about three or four weeks after mating, Wagner and Lazik, 1996).

The large variation in incubation periods at set temperatures means that it is inappropriate to calculate the Q_{10} values for *N. levis* incubation period. The Q_{10} determined for *U. milii* (2.27) is within the published range for small geckos
(1.95 – 3.35 at similar temperatures, Snyder, 1979).

5.4.7 Conclusions

Nephrurus and *Underwoodisaurus* species can improve their offspring survival by choice of suitable nesting sites. It is not known how geckos determine what is a suitable nesting site, but choosing sites that are adjacent to or beneath objects such as rocks or vegetation, will at least sometimes result in increased egg protection against extremes of temperature and water potential.

The degree of calcification of and eggshell conductance in *Nephrurus* and in *Underwoodisaurus* species allows some uptake of water to increase the reservoir buffer against possible desiccation, but also reduces the rate at which water is lost. Whether coating eggs with sand after laying influences desiccation-resistance has not been proven. The significance of shell pores in embryonic respiration has not been demonstrated, but their range in size, low density and also the lack of organization of the calcareous layer suggests they may not be the only means of gas exchange across the shell.

Gecko species laying flexible-shelled eggs lay relatively larger eggs than those laying rigid-shelled eggs, however arid-adapted diplodactyline species lay even larger eggs than mesic diplodactyline species. The adaptation of increased egg size producing two large offspring in arid-adapted diplodactyline species (combined with multiple clutching per season) is superimposed on the phylogenetic constraint of small clutch size to produce a closer-to-optimum strategy for ensuring survival in arid regions. Large egg size also increases resistance to aridity by increasing the egg water reservoir. The sexual size dimorphism with

larger females in *Nephrurus* species correlates with the trend towards larger egg size. Also the production of elongated eggs in diplodactyline species allows eggs to acquire a larger water reservoir for increased desiccation resistance. The significance of the occasional one-egg clutches is not clear, but their increased size may also enhance desiccation resistance.

The large variance in most of the parameters measured, such as *e.g.* size, incubation duration and time of laying, suggest a plasticity of reproductive strategy that could be a response to highly variable ecological environments in Australia.

Chapter 6

Biochemistry and Energetics of *Nephrurus* and *Underwoodisaurus* Eggs

6.1 Introduction

6.1.1 Egg Metabolism

The biochemistry and metabolism of avian eggs have been studied in considerable detail (*e.g.,* Vleck et al., 1980; Brammel, 1984; White, 1991). More recently, studies of the composition of reptilian (excluding Aves) eggs and comparisons with avian eggs have also been made (*e.g.,* Noble et al., 1990; Noble, 1991; Palmer and Guillette, 1991; Speake and Thompson, 1999). Emerging from a better understanding of egg metabolism arose concepts such as 'incubation energetics' and 'reproductive effort', which form important aspects of the evolution of life history strategies (*e.g.,* Tinkle, 1969; Pianka and Parker, 1975; Vleck, 1978; Vitt and Congdon, 1978; Turner, 1991). The reproductive energetics of the eggs of a number of reptilian species has been examined (*e.g.,* Tracy, and Snell, 1985; Thompson, 1987; Vleck and Hoyt, 1991; Noble, 1991; Schwarzkopf, 1994), but little information on the chemistry and metabolism of gecko eggs is available. Nevertheless, the major aspects of the biochemistry of gecko eggs would be expected to be similar to the findings in other reptiles (White et al., 1973).

A study of the energetics of reproduction and the relationships between body size and reproductive effort of geckos shows 'extreme conservatism' in relative clutch mass (RCM) among lineages of squamate reptiles (Doughty, 1996). The dry mass-specific energetic cost of embryonic development of the gekkonine gecko *Christinus marmoratus* (11.3 kJg^{-1}) is less than in other lizards (Thompson and Russell, 1999b). The current study makes a contribution to an understanding of gecko egg metabolism by testing the assumption that lipid provides most of the energy to fuel embryogenesis and by providing biochemical and metabolic data to confirm the pattern of development in *Nephrurus* and *Underwoodisaurus* embryos.

6.1.2 Embryonic Patterns of Oxygen Consumption

Ontogenetic changes in rates of O_2 consumption in embryonic birds show three distinct patterns. 1. An exponential pattern of VO_2 occurs in altricial birds. 2. A sigmoidal pattern occurs in most precocial birds (Vleck, C. M. et al., 1980). 3. In some ratite birds, the precocial pattern is modified with reduced oxygen consumption just prior to hatching, giving a 'peaked' oxygen consumption pattern (Hoyt et al., 1978; Vleck, C. M. et al., 1980; Cannon et al., 1986). These three modes of oxygen consumption are also present in reptiles (excluding Aves) (Thompson, 1989). In the 'peaked' mode, maximum oxygen consumption occurs at about 80 % of incubation period, followed by a steady decline prior to hatching (Thompson, 1989). Some turtles and the tuatara have a sigmoidal (or logistic) pattern of oxygen consumption (Ackerman, 1981; Thompson, 1985; Thompson, 1989) during embryonic development, whereas snakes have an exponential pattern (Clark, 1953; Dmi'el, 1970; Black et al., 1984; Gettinger et al., 1984). The significance of these differences is not known, although the peaked' and sigmoidal modes of oxygen consumption in embryonic reptiles may allow synchronised hatching with a resulting benefit of predators being swamped by large numbers of hatchlings (Carr, 1967; Thompson, 1988, 1989.).

All oviparous reptiles hatch at a relatively precocial stage of development, so the question arises as to why there are distinctly different modes of embryonic oxygen consumption among taxa (Thompson, 1989). Oxygen consumption governs metabolic rate in aerobic metabolism (Schmidt-Nielsen, 1997), so changes in embryonic respiration signal changes in energetics of development. Few data are available for geckos, but the energy content of a gecko egg (species unspecified) gives a value of 26.5 $kJ.g^{-1}$ dry mass (Withers, 1992), indicating a large lipid content. Many geckos have a large RCM, implying a large energy investment in their offspring (Appendix 6, Table A6.9). The RCM investment per progeny for a single clutch of *Nephrurus amyae* (as *N. asper*) eggs is 24.8 % of non-gravid maternal mass (Bedford and Christian, 1993); however, no analysis of energy content was given. Australian geckos have relatively high reproductive energy expenditure per progeny (Christian and Bedford, 1993). Embryonic metabolism of a gekkonine, *Christinus* (as *Phyllodactylus) marmoratus* is based on protein (60 %) and lipid (40 %), with an energy equivalent of 19.2 kJL^{-1} (Thompson and Russell, 1999b).

6.1.3 Maternal Reproductive Investment

The major component of reproductive investment by female geckos is the production of eggs (e.g., Charnov, 1982; Read, 1999). The proteins and energy-rich lipids in eggs represent a large energetic output by the female that requires regular replacement at every reproductive event. There are also numerous incidental energetic costs involved in reproduction, including the production or acquisition and transfer to the egg of approximately 40-50 essential nutrients (depending on species and stage of development, Nutrition Research Council, 1994; Endicott, 1993; Wardlaw and Insel, 1999). Incidental costs of reproduction, such as increased foraging or changes in maternal metabolism, may be variable and often difficult to quantify, but analyses of egg composition will approximate the amount of energy required in the production of each clutch.

Lipids form the major nutritional component of avian (Speake, Noble and Murray, 1998) and reptilian eggs (to a lesser extent) (Speake and Thompson, 1999). Increased incubation temperature reduces the incubation period for reptilian eggs (Packard, G. C. and Packard, M. J., 1988), and critical minima and maxima circumscribe the conditions of embryogenesis. Incubation of eggs at two or more temperatures allows a calculation of the Q_{10} of embryonic development that can then be used to compare the temperature effects on metabolism of *Nephrurus* and *Underwoodisaurus* geckos.

The net transfer of oxygen, carbon dioxide and water vapour across an eggshell or within an embryo is largely by diffusion and is, therefore, dependent on the presence of concentration gradients (Paganelli, 1991; Deeming and Thompson, 1991). Direct measurement of oxygen and carbon dioxide conductances are complex procedures, so they have often been estimated from measured values of water vapour flux (Ar et al., 1977). Eggshell conductances have been measured or calculated for a number of reptilian eggs, including those of crocodiles, turtles, lizards and snakes (Lynn and von Brand, 1945; Muth, 1980; Ackerman, 1981; Black et al., 1984; Gettinger et al., 1984; Ackerman et al., 1985; Thompson, 1985; Thompson, 1989; Thompson and Stewart, 1997; Thompson and Russell, 1998, 1999b). Eggshell conductance to water vapour and oxygen varies with shell thickness and structure, as well as microenvironmental factors that influence gas tensions and water potential.

6.1.4 Aims

The major aim of this part of the study was to describe the physical, chemical and respiratory characteristics of *Nephrurus* and *Underwoodisaurus* eggs during incubation, and relate these findings to the reproductive strategies employed. I measured the rates of oxygen consumption throughout incubation and quantified the water, lipid, protein, carbohydrate and ash content of new-laid eggs and eggs near hatching. From these measurements I determined the total energy content and energy density in normal eggs and the changes occurring during embryonic development of both *Nephrurus* and *Underwoodisaurus* eggs. The questions addressed are 1. What are the characteristics of maternal reproductive investment in *Nephrurus* and *Underwoodisaurus* reproduction? 2. Is there any difference in the proportions of lipids, proteins and water in *Nephrurus* and *Underwoodisaurus* eggs? 3. What is the pattern of protein, lipid and carbohydrate consumption during embryonic development? 4. What is the pattern of embryonic oxygen consumption in developing *Nephrurus* and *Underwoodisaurus* eggs?

The specific aims relating to these questions are as follows:

1. To determine the gas exchange characteristics of gecko eggs during embryonic development. By comparing the two groups, any differences would give information on conductance of the eggshell.
2. To determine the effects of substrate water potential on gas exchange and incubation of gecko eggs. This will help determine whether arid gecko species are less affected by changes in substrate water potential than mesic geckos.
3. To measure the effects of incubation temperature on gas exchange characteristics and incubation periods of gecko eggs and thus further define reproductive strategies and possible variations related to life history characteristics.
4. To estimate the energetic cost of embryonic development by measuring oxygen consumption ($VO_{2(tot)}$) of the eggs during incubation, and also by measuring lipid and protein content of freshly laid eggs and eggs near hatching. These measurements will determine whether lipids are the major source of embryonic energy and also whether the reproductive strategy involves the variation of metabolic rate to cope with possibly adverse conditions.

6.2 Methods

6.2.1 Egg Incubation and Sampling

To measure embryonic metabolism, gecko eggs were maintained in insulated laboratory incubators (Qualtex ST) at 25 or 30 ± 1 °C (Chapter 5, Appendix 6). These temperatures were chosen to be within the limits of tolerance for all the species examined and not likely to induce any abnormal embryonic changes (e.g., Wagner, 1980; Seufer, 1991; Grover, 1994; Henkel & Schmidt 1995). The experimental design also allows analysis of whether temperature results in changes in total oxygen consumption ($VO_{2\,(tot)}$), which would indicate efficiency of offspring production. *Nephrurus levis* and *U. milii* were chosen as representative species for the two genera, and a small number of eggs from *N. deleani* and other species were also analysed for comparative purposes.

All available gecko eggs were used in analyses. Damaged eggs were retained for observations of embryonic development and for composition analysis. Infertile eggs were either frozen for composition analysis or used for determination of initial desiccation rates (*i.e.* prior to the period in later embryonic development when the embryo may exert some control over egg water (Chapter 5). Thirty-two *U. milii*, ten *N. levis* and three *N. deleani* eggs were chosen at random for various analyses. Each egg was measured to the nearest 0.1 mm using electronic digital callipers, weighed to the nearest 1.0 mg using an electronic Sartorius analytic balance, and sealed in a labelled transparent plastic film container and stored at -20 °C for later analysis. Ten of these eggs were chosen at random and separated into shell and contents for wet and dry shell measurements and egg contents analysis. In addition eight late-stage *U. milii* embryos and two *N. levis* embryos were also separated from the eggshells, weighed and frozen prior to analysis. Fresh wet eggshells of eggs that completed incubation were weighed and the shells reweighed after washing and again after freeze drying in a Christ freeze drier, model Alpha 2-3. Twelve post-hatching egg residues (usually only a few microlitres each) were analysed by the Bayer Diagnostics, Ames urinalysis dipstick (colorimetric) method and five residue samples were combined for analysis by standard analytical techniques (see below). The egg samples, mature embryos and egg residues were lyophilised in the freeze drier, and water content was determined by subtraction. Partial egg samples (N = 41) (excluding shell) of approximately 20 mg were used for ash content (Section 6.2.2), and duplicate freeze-dried samples (N = 37) were

taken for determination of total nitrogen to estimate protein content (Kerese, 1984; Section 6.2.4). Most of the remaining freeze-dried material was used for determination of total lipid (N = 46) content using chloroform/methanol extraction (Christie, 1984; Section 6.2.5). Fat bodies and tails were removed from mature embryos for separate analyses of lipid and protein content.

Chicken (*Gallus g. domesticus*) eggs were chosen as an example of avian eggs for comparison of composition with gecko eggs because their composition has been rigorously analysed and is similar to that of many other avian eggs (Stadelman and Cotterill, 1995; Speake and Thompson, 1999).

Eggshells were analysed separately for nitrogen, lipid and ash content. Because the shells tend to become crinkled and brittle when dried, the shell thickness was measured wet, using a NSK precision engineers micrometer, after soaking in water and cleaning under a dissecting microscope to remove adherent sand or membranes.

Tail volumes were calculated from measurements of tail length (L), maximum breadth (B) and maximum height (H) using the formula for the volume (V) of an ellipsoidal cone $V = \pi.(B/2).(L/2).H$. Fat bodies were categorised as being absent, small (<25 % of body cavity width) moderate (25 – 50 %), large (50 – 75 %) or very large (>75%) estimated by eye.

6.2.2 Water Content of Eggs, Eggshell and Embryos

The eggshells (N = 28 *N. levis*, N = 2 *N. levis*, N = 37 *U. milii*) and embryos (N = 2 *N. levis*, N = 10 *U. milii*) were later desiccated to determine water content, either by freeze-drying or in an oven at 100 °C until weight was constant. Eggshells were prepared as for ashing, (Chapter 5; Appendix 6). Shells, egg contents and embryos were weighed fresh, after desiccation and again after ashing. Water content was determined from wet and dry weights by subtraction. Ash content was defined as the residue remaining after heating samples at ~ 400 °C for at least 30 minutes. Ash was weighed only after samples had cooled to room temperature.

6.2.3 Carbohydrate Analysis

Twenty fresh wet egg samples (N = 6 *N. levis,* N = 14 *U. milii*) and five post-hatching egg residues (N = 2 *N. levis,* N = 3 *U. milii*) were analysed for reducing sugars and for ketones,

using Bayer colorimetric dipsticks, Multistix (as used for urinalysis) and the reference colour chart provided by the manufacturer (Henry, 1986). The dipstick reagent surface was placed on the wet surface of the egg contents or on the wet internal surface of the eggshell after hatching or directly on the yolk residue material if present.

6.2.4 Nitrogen Analysis

Indirect protein estimations were made using the standard Kjeldahl method of analysis of nitrogen in egg samples (Mann and Saunders, 1967; Kane, 1984). Duplicate freeze dried samples (N = 8 *N. levis*, N = 2 *N. deleani*, N = 27 *U. milii*), were analysed using an automated Tecator System Digestion Unit 1009 and a Kjeltec System 1026 Distilling Unit. Samples were added to large glass boiling tubes together with a Kjeldahl selenium catalyst tablet (Labchem A, #2206-1000) and 5 mL of concentrated (98 %) sulphuric acid. The mixture was heated to 400 °C for 90 minutes and cooled. Excess NaOH (40 %) was automatically added prior to distillation. The distillate was collected in a flask containing 25 mL of 4 % boric acid, automatically titrated with 1M HCl and the nitrogen content of the original sample calculated (Morrison and Boyd, 1999). The protein content in the sample was calculated from the nitrogen values using the standard Kjeldahl conversion factor of 6.25 for food proteins (Kane, 1984).

6.2.5 Lipid Analysis

The standard acid hydrolysis method of lipid analysis (Christie, 1984) was used. Clean glassware was preweighed at room temperature using a Mettler H35 AR analytic balance. Solvents used were high-pressure liquid chromatography grade or better. Lipid analyses were done after other analyses using all of the remaining material to maximise the sample size (N = 10 *N. levis*, N = 2 *N. deleani*, N = 34
U. milii), and hence increase accuracy and precision of results.

The energetic density of gecko egg lipids and proteins as measured by chemical analysis was determined using published values for similar compounds; 39.3 $kJ.g^{-1}$ for animal fats; 18.0 $kJ.g^{-1}$ for proteins, where urea is the metabolic end-product (Schmidt-Nielsen, 1997).

6.2.6 Oxygen Consumption Rates

Oxygen consumption of eggs was measured periodically throughout incubation using Warburg manometers at 25 ± 0.01 °C or at 30 ± 0.01 °C. Laboratory grade mercury-in-glass thermometers, reading to ± 0.05 °C, were used to monitor ambient and water bath temperatures. Because of the large manometer chambers necessary for housing large *Nephrurus* spp. eggs, there was a longer than normal time lag in equilibration of the system. An aneroid barometer calibrated against a mercury barometer was used to measure barometric pressure. Two control chambers, each set up as the experimental chambers except without an egg, were used during each experiment because of the hysteresis of temperature and pressure changes in large manometer chambers. An excess of 10 % KOH was used as a CO_2 absorber. The volumes of the glass manometer chambers were determined to a precision of 0.01 mL by filling with water at 25 °C and weighing. Brodie's solution (an aqueous solution containing detergent and methylene blue) was used as a manometric fluid. Eggs were supported in the chamber on modified polythene centrifuge tubes with attached recessed caps. The recessed cap was used to hold the egg and the tube contained the KOH in a small wad of cotton wool. The CO_2 was absorbed via numerous small holes drilled in the tube. Glass-to-glass joints were sealed using petroleum jelly and the chambers were checked for leaks by running them empty for several days and making sure they were still dry inside. Cyclohexanone was used to seal PVC joints in tubing.

Recently laid eggs (N = 66) were left in the manometers overnight for up to 24 hours to gain a reasonable manometry pressure change. On completion the temperature, barometric pressure and manometer pressure changes were recorded and the taps opened to the atmosphere prior to returning the eggs to the incubator. The rate of oxygen consumption was then calculated using the pressure change in the manometer, the volume of the manometer and the duration of the experiment (Novak and Mitchison, 1986). The duration of incubation was expressed as days from hatching to enable a better comparison of oxygen consumption rates in different eggs. The rate of oxygen consumption (VO_2) was calculated as mL per day and then plotted against days prior to hatching. An exponential line was fitted to the data and the area under the curve integrated to provide a value for the total oxygen consumption under the specified conditions in the four groups of eggs (incubation at 25 °C, 30 °C, -100 kPa, -450 kPa).

Some Warburg manometry experiments were run continuously for several days to determine whether or not there was any diurnal rhythm to the oxygen consumption pattern. This was achieved by placing a 60W lamp near the water bath that was connected to a timer; the photoperiod was adjusted to 12 hours on and 12 hours off to produce a distinct diurnal rhythm. Eggs were weighed before and after respirometry to determine if there were any significant changes in mass over these long experimental periods. Eggs were also candled to determine progress of embryonic development. Embryos were also observed for spontaneous or triggered movement as the hatching time approached.

6.3 Results

6.3.1 Egg Fluids

Fresh laid gecko eggs show no distinct compartmentalised yolk and no albumen layer under the shell. When a gecko eggshell is ruptured, a viscous, creamy coloured fluid is released, the amount depending on the internal pressure, which varies with amount of water uptake and the elasticity of the shell. The egg fluid contains numerous microscopic lipid droplets. At hatching (and sometimes prior to hatching) a clear viscous fluid, which is distinct from the yolk residue (probably allantoic fluid), may leak out of the egg. Transillumination of gecko eggs within the first few days of incubation shows the growing embryonic disc with a clear fluid around the embryo and which soon extends beyond the sinus terminalis and presumably envelops the whole egg (when it is no longer visible as a distinct zone by transillumination) (Chapter 5). Observations of four hatchlings that evacuated their cloacae soon after hatching showed only minute amounts of white insoluble material (presumed uric acid) in clear watery fluid.

There was no difference in the proportions of water found in *Nephrurus* and *Underwoodisaurus* eggs at laying (79.6% for *N. levis* and 80.8% for *U. milii*, $p = > 0.05$, Tables 6.1, 6.2). Also, the protein and lipid concentrations of infertile and fertile eggs were not significantly different, so the results were combined for analysis.

6.3.2 *Nephrurus levis* Eggs

Egg data were collected for 46 *N. levis* eggs laid in 21 clutches of two eggs and four clutches of one egg (Table 6.1, Appendix 6, Table A6.5). Two clutches of two eggs were probably retained by the female longer than usual (see section 3.3.6). The mean value for

water content was 79.6 ± 7.9 % (N = 11), protein (54.6 ± 6.4 % of dry mass, N = 11) and lipid (32.7 ± 6.4 % of dry mass, N = 12) form the bulk of the freshly laid egg dry mass. The ash content of egg (excluding shell) is 6.62 ± 1.05 % of dry mass (N = 7). The eggshell is 1.73 ± 0.49 % (N = 28) of wet mass or 10.4 ± 3.00 % of whole egg dry mass.

Table 6.1 *Nephrurus levis* egg and hatchling composition

CHARACTER	MEAN	RANGE	STD	N
Initial egg mass (g)	2.48	1.66-3.31	0.40	46
Prehatching egg mass (g)	3.24	2.21-4.71	0.68	20
Initial egg water %	79.6	65.6-92.0	7.89	11
Whole egg dry mass (mg)	414	194-802	204	11
Egg dry mass % of initial mass*	14.7	5.74-20.5	5.94	6
Clean dried shell (mg)**	42.9	27.8-79.8	12.1	28
Dry mass excluding shell (mg)	379	155-769	207	11
Egg protein % dry mass*	54.6	47.6-63.8	6.35	11
Protein energy (kJ/egg)	4.28	2.01-6.86	1.56	8
Egg lipid % dry mass	32.7	19.5-42.3	6.37	12
Lipid kJ/egg	5.72	2.23-10.5	3.07	11
Lipid + protein $kJ.g^{-1}$	22.2	14.4-27.1	3.55	11
Lipid + protein kJ/egg	11.8	5.92-17.4	4.37	6
Egg ash % of dry mass	6.62	4.90-8.38	1.05	7
Relative clutch mass (%)	29.5	24.8-37.2	4.51	7
Hatchling mass (g)	1.85	0.876-2.40	0.413	19
HM as % initial egg mass	82.5	69.0-91.0	7.28	15
Residuum %***	7.13	1.92-13.2	3.47	11

* Initial egg mass minus dry shell mass.

** Includes shells from hatched and unhatched eggs

*** The residuum is calculated by subtracting the percentage values for proteins, lipids and ash from 100 %.

6.3.3 *Underwoodisaurus milii* Eggs

Egg data were collected for 83 *U. milii* eggs laid in 39 clutches of two eggs and five clutches of one egg (Table 6.2, Appendix 6, Table A6.6). New-laid eggs consisted of 80.8 ± 6.5 % water, the dry mass showed 51.2 ± 4.1 % protein, (N = 27) and 35.7 ± 5.7 % lipid (N = 36) and 6.02 ± 2.38 % ash (excluding shell, N = 31). The eggshell was 2.81 ± 1.26 % (N = 36) of initial egg wet mass or 12.4 ± 4.7 % (N = 36) of whole egg dry mass.

Table 6.2 *Underwoodisaurus milii* egg and hatchling composition

CHARACTER	MEAN	RANGE	STD	N
Initial egg wet mass (g)	1.95	0.775-3.96	.477	81
Prehatching wet mass (g)	2.59	1.13-3.94	.604	45
Mass increase %	45.9	0.25-137	27.5	33
Surface area increase %	18.0	3.28-43.6	10.1	20
Initial egg water %	80.8	73.0-96.2	6.46	19
Whole egg dry mass (mg)	383	140-633	106	32
Egg dry mass % initial mass*	19.8	14.9-25.0	3.18	14
Clean dried shell (mg)	54.4	20.4-147	28.1	37
Dry mass excluding shell (mg)	358	102-574	104	17
Egg protein % dry mass*	51.2	42.0-58.0	4.28	27
Protein energy (kJ/egg)	3.35	2.15-5.13	0.87	12
Egg lipid % dry mass	35.7	28.6-52.7	5.72	36
Lipid kJ/egg	4.68	2.07-7.60	1.58	34
Lipid + protein $kJ.g^{-1}$	23.0	17.0-25.5	1.94	22
Lipid + protein kJ/egg	7.68	2.72-11.8	2.32	24
Egg ash % of dry mass	6.02	2.66-14.8	2.38	31
Relative clutch mass (%)	24.7	14.7-34.9	6.04	15
Hatchling mass (g)	1.67	0.97-2.05	0.29	33
HM as % initial egg mass	83.6	54.0-106	11.1	30
Residuum %**	8.59	0.36-24.5	5.72	21

* Initial egg wet mass minus dry shell mass.
** The residuum is calculated by subtracting the percentage values of mass for proteins, lipids and ash from 100 % (composition unknown but it probably includes complexed lipids and/or proteins).

6.3.4 *Nephrurus deleani* Eggs

Egg data were collected for eight *N. deleani* eggs laid in four clutches by four females (Table 6.3, Appendix 6). The mean value for water content was 78.1 ± 10.6 % (N = 3). Protein was 52.7 % of dry mass (N = 2) and lipid was 31.1 % of dry mass (N = 2) forming the bulk of the freshly laid egg. The eggshell was 1.24 % of wet mass or 4.2 % of whole egg dry mass. The ash content of egg contents (excluding shell) was 4.23 ± 0.191 % of dry mass (N = 3).

Table 6.3 *Nephrurus deleani* egg composition

CHARACTER	MEAN	RANGE	STD	N
Initial egg wet mass (g)	2.10	1.71-2.52	0.277	8
Prehatching egg wet mass (g)	2.48	2.35-2.69	0.132	5
Initial egg water %	78.1	71-90.2	10.6	3
Whole egg dry mass (mg)	616	549-682		2
Egg dry mass % initial mass*	21.9	9.8-29.0	10.6	3
Clean dried shell (mg)	26	20-32		2
Dry mass excluding shell (mg)	584	517-662		2
Lipid % of dry mass	31.1	30.9-31.3	0.307	3
Lipid kJ/egg	7.51	6.75-8.27		2
Protein % dry egg	52.7	48.5-55.1		2
Protein kJ/egg	5.50	4.62-6.38		2
Lipid + protein $kJ.g^{-1}$	22.0	21.5-22.5		2
Lipid + protein kJ/egg	13.0	11.4-14.6		2
Ash % of dry mass	4.23	2.74-4.61	.191	3
Relative clutch mass %	36.7	33.5-38.5	2.03	5

* Initial egg mass minus dry shell mass.

6.3.5 Lipid Composition of Fat bodies and Tails

Fat bodies in mature embryos appear oily and translucent at room temperature, indicating high lipid content (90 – 99 % of dry mass in *U. milii*). Because the embryonic tail lipids were present in very small amounts, the eight *U. milii* tails were combined to produce a mean lipid value of 16.0 % of tail dry mass (Table 6.4). The *N. levis* fat body lipid measurements were all within the range for *U. milii* (Table 6.5). The *N. levis* tail lipid measurement as % of dry mass was higher, at 19.5 %, compared to *U. milii*.

Table 6.4 Lipid content of *U. milii* embryo fat bodies and tails

	FAT BODY			TAIL		
	Wet mass (mg)	Dry mass (mg)	Lipid % dry mass	Wet mass (mg)	Dry mass (mg)	Lipid % dry mass
Mean	49.6±29	29.4±15	93.6±4.2	83.2±29	14.6±6.1	16.0
Range	17.9-90.3	14.0-51.9	90.3-99	40.0-128	7.8-26.0	(combined)
N	5	5	5	8	8	8

Table 6.5 Lipid content of *N. levis* embryo fat bodies and tail

	FAT BODY			TAIL		
	Wet mass (mg)	Dry mass (mg)	Lipid % dry mass	Wet mass (mg)	Dry mass (mg)	Lipid % dry mass
Mean	66.8	16.4	97.0	157	19.8	19.5
Range	35.6-98.0	16.4-17.1	95.9-98.0	-	-	-
N	2	2	2	1	1	1

Correlation of the tail volume and the abdominal fat body size in adults of three species showed low values (R^2 = 0.432, N = 138 in *N. laevissimus* (small tails), R^2 = 0.002, N = 11 in *N. vertebralis* (medium tails), R^2 = 0.237, N = 20, in *N. levis* (large tails). Lipid depletion of one site does not parallel depletion of the other.

6.3.6 Incubation and Rates of Oxygen Consumption

In both *N. levis* and *U. milii* there was no significant difference between egg incubation duration at –100 kPa and at –450 kPa water potential, (P = > 0.05). The same was true at both 25 °C and at 30 °C. Therefore the two incubation groups (-100 and –450 kPa) were combined to derive the mean oxygen consumption rates at each temperature.

Both *N. levis* and *U. milii* eggs show an approximately exponential pattern of increase in VO_2 during embryonic development at 25 °C and at 30 °C (Figures 6.1, 6.2). Incubation duration of *N. levis* eggs used in the Warburg manometry experiments at 30 °C was 49.6 ± 17.4 days (N = 9), approximately half of the 95.9 ± 15.1 days for eggs incubated at 25 °C (N = 8). For *U. milii*, incubation at 30 °C was 52.3 ± 2.9days (N = 4), approximately two thirds of the duration of incubation at 25 °C, 77.3 ± 7.7days (N = 12).

Nephrurus levis eggs incubated at 25 °C used more oxygen in total (in 108 days, N = 8) than the eggs incubated at 30 °C (in 60 days, N = 9). Similarly the *U. milii* eggs incubated at 25 °C used more oxygen in total (in 81 days, N = 12) than the eggs incubated at 30 °C (in 54 days, N = 4) (Table 6.6). Although *Nephrurus levis* eggs appear to use more energy per gram of dry hatchling mass than *U. milii* eggs during embryogenesis, the effects of retained eggs on measured metabolic rates of embryos cannot be determined from the available data.

There was no significant difference between nocturnal and diurnal oxygen consumption rates, so the longer-term experiments results were combined (Table 6.6). The total oxygen consumption during embryogenesis was converted to an energy equivalent assuming that 1.0L of oxygen releases 19.2kJ of energy (Thompson and Russell, 1999, 1999b) (Table 6.6). A comparison of gecko and avian (chicken) egg composition is made in Table 6.7.

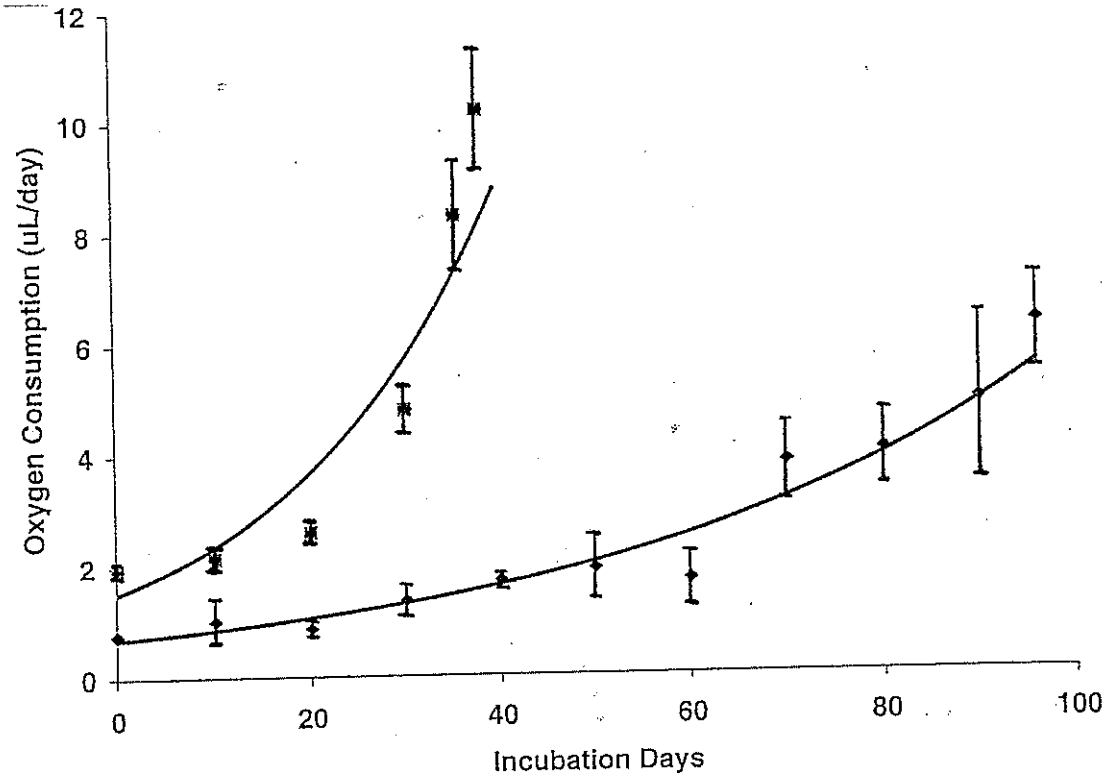

Figure 6.1 Patterns of embryonic oxygen consumption in *N. levis* at 30 °C (squares, N = 9 eggs), the fitted exponential line is given by $y = 6.441e^{0.0302x}$ and at 25 °C (diamonds, N = 8 eggs), the fitted exponential line is given by $y = 6.119e^{0.022x}$. Vertical bars represent ± one standard deviation.

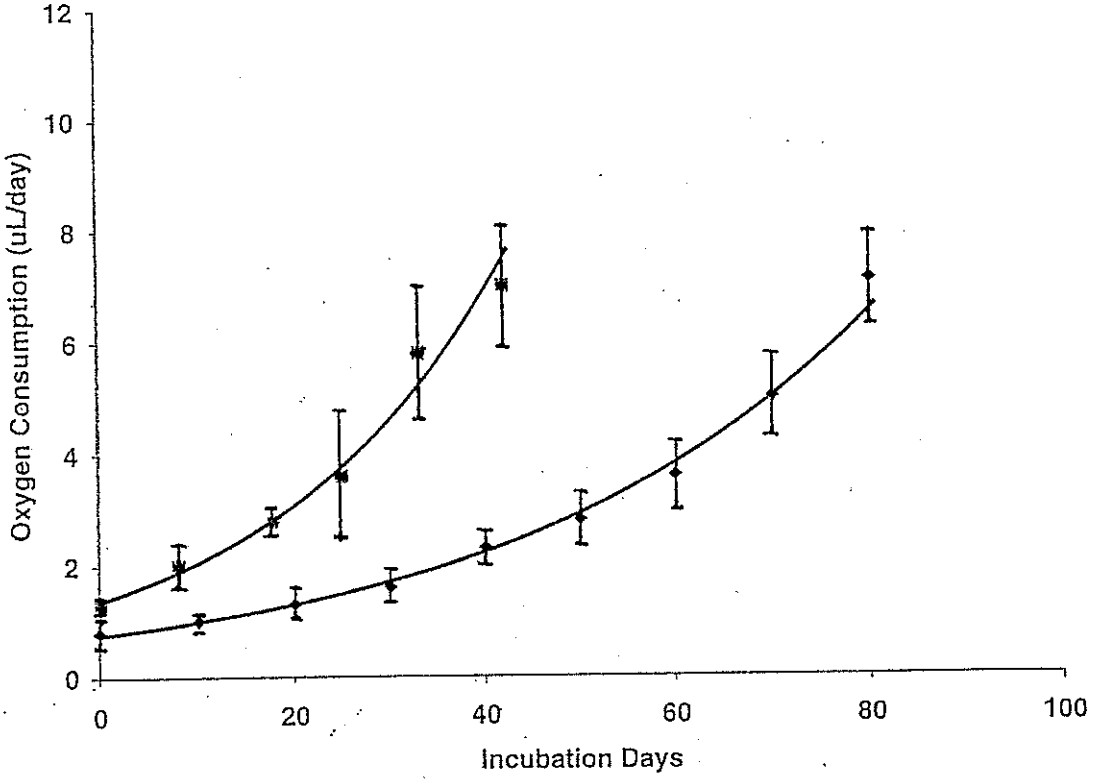

Figure 6.2 Patterns of embryonic oxygen consumption in *U. milii* at 30 °C (squares, N = 4 eggs), the fitted exponential line is given by $y = 8.472e^{0.0424x}$, and at 25 °C (diamonds, N = 12 eggs), the fitted exponential line is given by $y = 6.403e^{0.026x}$. Vertical bars represent ± one standard deviation.

Table 6.6 Mean energetic cost of embryonic development in *Nephrurus* and *Underwoodisaurus* eggs at 25 °C and 30 °C (N = number of eggs)*.

SPECIES	*N. levis*		*U. milii*	
Temperature (°C)	25	30	25	30
Incubation duration (days)	108±19.1	60.0±17.9	81.4±4.7	54.0±3.0
Total O_2 consumption (mL)	149.4±38.9	148.3±58.5	91.0±20.4	85.8±17.8
Energy equivalent (kJ)	2.868±0.75	2.847±1.12	1.747±0.25	1.648±0.23
Dry mass kJ/g	8.64±2.2	8.57±3.4	5.82±0.83	5.49±0.79
N	8	9	12	4

* Because there was no significant difference between the nocturnal and diurnal oxygen consumption rates the results of the longer-term experiments have been combined in this table.

Note increased variance in incubation duration in *N. levis* possibly due to egg retention.

Table 6.7 Shell-free composition of chicken and gecko eggs (weight for weight). N = number of eggs, values ± one standard deviation.

	WATER %	PROTEIN % dry mass	LIPID % dry mass	BALANCE % dry mass	ENERGY $kJ.g^{-1}$
Chicken egg*	74.0	49.3	44.2	7.0	26.3
N. levis (N=11)	79.6±7.9	54.8±7.2	31.9±6.7	13.3	22.4±1.4#
U. milii (N=19)	80.4±6.5	51.1±4.2	35.2±7.2	13.7	23.1±1.4#

The BALANCE is the sample proportion unaccounted for by lipid and protein components, which consists of ash and other unresolved components (Morrison and Boyd, 1987; Stadelman and Cotterill, 1995).

$kJ.g^{-1}$ dry mass of whole, shell-less egg, calculated from lipid and protein content using standard conversion factors (Schmidt-Nielsen, 1996).

* Chicken egg values derived from Suhrid Geigy, (1964).

No ketone bodies were found in eggs or extraembryonic fluids or residues and only a slight trace (*i.e.*, < 0.25 %) of reducing sugars was found associated with some residual yolk material.

Embryos became increasingly active close to the hatching time. Several eggs were monitored continuously through the hatching process, all showing a further increase in oxygen consumption beyond the prehatching surge. The hatching gecko struggles

vigorously to force the egg tooth through the eggshell and makes lateral slashing movements of the snout (accompanied by undulating movements of the body and tail) to open up a sufficient opening, to breathe air initially and later to escape the egg.

6.4 Discussion

6.4.1 Comparison of Composition of Avian and Gecko Eggs

Gecko (*Nephrurus* and *Underwoodisaurus*) and chicken egg compositions (Suhrid Geigy, 1964) are generally similar but gecko eggs have more water, more protein, less lipid and lower energy density in terms of dry mass values (Table 6.7).

Although the mean value for proportion of water in gecko eggs at oviposition is larger than for chicken eggs (Table 6.7), this difference may not be statistically significant (untestable due to lack of chicken egg data). The increased gecko egg water content found in later embryonic development probably is significantly different to that found in avian eggs. The extra water is presumably there as a safety factor to reduce the risks of desiccation (Badham, 1971). The higher proportion of dry-mass lipid in avian eggs may be related to the need to reduce egg mass to a minimum so that flight is not compromised. As most vertebrates have similar lean-mass body compositions, the similarity of the protein proportions is not surprising. Gecko eggs are similar in composition to skink eggs (Speake et al., 1999; Speake and Thompson, 2000) suggesting that the energetics of avian eggs may differ from squamates, although more reptilian egg composition data are required for confirmation.

6.4.2 Egg Water Content

Of fundamental significance, water is essential in the diffusion and circulation of gases, nutrients and metabolites within the egg and embryo (Campbell et al., 1999). Water is also essential for biochemical processes, including lipid and protein metabolism (White et al., 1984). Net water uptake by flexible-shelled reptile eggs continues throughout embryonic development, even though the percentage of embryonic water decreases throughout development (Thompson and Russell, 1999b). This supports the idea that the functions of the egg water are both internal and external to the embryo, (Packard et al., 1977). One external function is the dilution of increasing amounts of nitrogenous waste in the allantois

(Campbell et al., 1999).

The smaller proportion of water in avian eggs compared to squamate eggs is partly accounted for by the higher proportion of lipids. The near-constant proportions of water found in large and small eggs of squamates and in both rigid-shelled (Thompson and Russell, 1999b) and recently laid flexible-shelled eggs (Thompson and Russell, 1999; Thompson et al., 1999) suggest that there is no increased water reservoir in the eggs of rigid-shelled eggs. This also suggests that any increased water reservoir effect in flexible-shelled eggs is achieved by water absorption after oviposition. The range of variation found in water content in both *Nephrurus* and *Underwoodisaurus* eggs suggests that although there is a minimum water requirement (present at oviposition), there is considerable plasticity in this character.

6.4.3 Egg and Embryo Lipids and Carbohydrates

Lipids form the major nutritional component of birds' eggs (Noble, 1991; Noble and Speake, 1997; Speake and Thompson, 2000). The lipids in a 50 g chicken (*Gallus g. domesticus*) egg constitute approximately 5.1 g (~11.5 % of wet mass or 44 % of dry mass) (Suhrid Geigy, 1964). My findings are that the relative proportion of lipids in gecko eggs is only about 60 % of that in hens' eggs (33 % of the dry mass). Nevertheless there is still a major reliance on high-energy lipids for embryonic development of lizards (Thompson and Russell, 1999b; Speake and Thompson, 2000). A proportion of the original lipid in *Nephrurus* (12.9 %) and *Underwoodisaurus* (21.7 %) eggs is incorporated into the embryonic abdominal fat body prior to hatching. The caudal lipids are less in hatchling *U. milii* compared to the adults, where lipid reserves are found both in the abdominal fat bodies and in the tail. The high level of lipids in the fat bodies (93.6 % in *U. milii* and 95.9 % in *N. levis*) (Tables 6.4, 6.5) indicates the importance of this organ as an energy reservoir. The mass of the abdominal fat bodies of mature embryos varies between 17.9-90.3mg (N = 8) in *U. milii* (~2-5 % of hatchling wet mass) and 35.6 - 66.8 mg (N = 2) in *N. levis* (~2-3 % of hatchling wet mass). The small fat bodies found in some hatchlings may result from either low maternal energetic investment in that egg, or high total energy expenditure of the embryo during incubation *e.g.,* due to incubation at a lower temperature.

The tails of *U. milii* appear swollen on hatching, but they are cylindrical in shape and not depressed (or laterally swollen) as in adult tails with stored lipids. The tails of *N. levis* hatchlings are depressed but relatively narrow compared to adult tails. The tails of mature embryos of *U. milii* contain 16.0 % of the dry mass as lipid (N = 8, pooled for lipid analysis); this compares to 19.5 % in *N. levis* (N = 1). Analysis of the rest of the specimens (minus tails and fat bodies) gave similar results to the tail analyses with a mean value of 19.9 % lipid in mature *U. milii* embryos. These data show that the fat body is the major lipid storage organ and that nearly half (44.7 %) of the original egg lipid is utilised in embryonic development.

No reducing sugars were found in egg residues, and egg yolks either lacked or contained a slight trace (*i.e.*, < 0.25 %). A normal blood glucose level is expected in the embryo. This is confirmed by the lack of ketone bodies in the egg residue fluids, which would be expected if metabolism were largely lipid/protein based (White, et al., 1984). Gluconeogenesis normally occurs in hepatic and renal tissues (Sackheim and Lehman, 1981) and possibly the embryonic precursors, so that there would be no necessity for yolk carbohydrate. Fresh chicken eggs contain approximately 0.7 % of wet mass as carbohydrate (type unspecified) (Suhrid Geigy, 1964). The lack of reducing sugars in the extraembryonic gecko egg fluids suggests that the embryonic metabolic energy is contained largely in the proteins and lipids, as found by Vleck and Hoyt (1991).

The lack of correlation between tail volume and abdominal fat body size in adults of three species of *Nephrurus* with large medium and small tails together with the finding that lipid depletion of one site does not parallel depletion of the other suggests that the lipid in the two sites may be utilised differently or for differing purposes. Females use significant lipid amounts in egg formation but the amount supplied to embryos is not revealed as lipid storage in hatchling tails of *U. milii* but possibly some storage in *N. levis* tails.

The correlation of the tail volume and the abdominal fat body size in adults of three species of *Nephrurus* showed low correlation ($R^2 = 0.432$, N = 138 in *N. laevissimus* (small tails), $R^2 = 0.002$, N = 11 in *N. vertebralis* (medium tails), $R^2 = 0.237$, N = 20, in *N. levis* (large tails). Lipid depletion of one site does not parallel depletion of the other. Correlate adults & embryos?

6.4.4 Egg and Embryo Proteins and Ash

The fraction of protein found in *Nephrurus* and *Underwoodisaurus* eggs is well within the range (40-68 % of dry mass) reported for lizard eggs (Ballinger and Clark, 1973; Hadley and Christie, 1974; Vitt, 1978b; Thompson et al., 1999). There is a slightly higher proportion of dry mass protein in the larger *N. levis* eggs compared to the *Underwoodisaurus* eggs ($t_{(2),36} = 2.038$, $P = 0.049$). If there is a selective advantage in producing larger offspring in arid geckos, then it might be expected that more protein at a higher concentration would be found in *Nephrurus* eggs; however, more data are needed to confirm any relationship.

A proportion of the dry mass egg contents (7.12 ± 3.69 % in *N. levis*, N = 9, and 8.59 ± 5.72 % in *U. milii*, N = 21) was not accounted for by the combined mass of the lipid, protein and ash. A similar residual fraction has been found by others (D. T. Booth, personal communication), but its composition is uncertain. This residual fraction could be partly artifactual *e.g.,* there are possible losses in filtration, and of volatiles during heating. Also, part of the residual fraction may result from the presence of small amounts of compounds such as carotenoids, cholesterol, butyrate, succinate, lactate, vitamins, and complexed enzymes; certain components of which may not be fully accounted for by the current methods (Christie, 1984; Dobush et al., 1985; Stadelman and Cotterill, 1995).

The usually clear but slightly viscous residues left in the egg shell after hatching contains very little protein, and no lipids or reducing sugars (Badham, 1971, Deeming, 1989) indicating efficient usage of resources. Rarely, a small amount of egg yolk was found remaining in the eggshell after hatching, in which cases small amounts of lipid and protein were found. The residual post-hatching egg proteins and lipids have similar concentrations to those found in whole eggs, suggesting either, that both provision (in egg formation) and consumption (by the embryo) of these nutrients is well balanced, or that the embryo uses whatever nutrients are available regardless of concentration (Sinervo, 1990, 1994). The fact that large and small embryos hatch from eggs with minimal nutritional residues suggests that the hatchling will emerge when sufficiently mature, whether large or small. The limited *N. deleani* protein analyses are similar to those for *N. levis*.

Chicken egg contents have 1.7 – 4.0 % ash (Suhrid Geigy, 1964; Phillipson, 1964; Stadelman and Cotterill, 1995), but it might be expected that a parchment-shelled reptile egg would have more because a developing squamate embryo requires about ten times the amount of calcium compared to a similar sized avian embryo (Jenkins and Simkiss, 1968). The relative ash content of gecko eggs is greater than in hens' eggs (Table 6.7); however, ash measurements are variable in both groups.

6.4.5 Embryonic Energetics

The energy density of gecko eggs might be expected *a priori* to be higher than for avian eggs, because lizard eggs do not have a separate albumen component (Badham, 1971; Palmer and Guillette, 1991) and therefore a higher proportion of (yolk) lipids compared to chicken eggs. However, avian eggs have a higher energy density than squamate eggs because they contain more lipid (Speake and Thompson, 1999). A 50 g chicken egg has an energy content of approximately 23.36 $kJ.g^{-1}$ of dry mass (Passmore, 19673) *i.e.*, approximately 301 kJ for the 44 g of egg contents, assuming the shell is 12 % of total egg mass (Anderson, 1994).

The mean dry mass energy densities were almost identical in the eggs of all three species analysed, ranging from 22.0 $kJ.g^{-1}$ in *N. deleani* to 23.0 $kJ.g^{-1}$ in *U. milii* (as calculated from protein and lipid content). This compares with a mean of 26.8 ± 0.89 $kJ.g^{-1}$ for 29 species of lizards (including two gecko species) (Ballinger and Clark, 1983; Vitt, 1978b; Vitt and Oharmt, 1975; Booth and Thompson, 1992; Withers, 1992; Thompson and Russell, 1999). The means for egg energy density were not significantly different in *N. levis* (22.2 ± 3.55 $kJ.g^{-1}$, N = 10) and in *U. milii* (23.0 ± 1.94 $kJ.g^{-1}$, N = 22), (P = >0.05). The reason for the large ranges in energy density is not known. The use of infertile eggs is unlikely to have contributed to an increased range because the mean value is close to the median of the range (infertile eggs, if different, would be expected to have a low energy density rather than high and skew results).

The mean energy density for *N. levis* eggs (22.2 ± 3.6 $kJ.g^{-1}$) is not significantly different from *U. milii* eggs (23.0 ± 1.9 $kJ.g^{-1}$) (P > 0.05). Both values are within the published range for lizard eggs (18.2 ± 0.7 $kJ.g^{-1}$ for *Morethia boulengeri* (Thompson and Russell, 1999) to 28.1 $kJ.g^{-1}$ for *Cnemidophorus tigris* (Vitt, 1978b)). The energy density figures for *N. levis*

and *U. milii* eggs are 15% lower than for the eublepharid gecko *Coleonyx variegatus* of 26.5 $kJ.g^{-1}$ (Vitt, 1978b).

The embryonic energy consumption of some skinks is approximately equally derived from lipids and proteins (Thompson and Russell, 1999). The pattern in *Nephrurus* and *Underwoodisaurus* geckos probably is similar to that in skinks because, although lipids contain approximately 2.14 times the energy density of proteins, they are present as only 38.6 % of the total mass of protein and lipids. This gives a calculated respiratory exchange ratio of 0.762 (assuming proportional utilisation of proteins and lipids and insignificant carbohydrate metabolism), which is identical to a measured value of 0.76 for each of two species of skink (*Morethia boulengeri* and *Morethia adelaidensis*) (Thompson and Russell, 1999).

6.4.6 Respirometry of Embryos

Both *N. levis* and *U. milii* embryos showed an exponential pattern of oxygen consumption during development under all four experimental conditions. This pattern is also found in altricial birds (Vleck and Vleck, 1977) and other lizards (Thompson and Stewart, 1997). Just prior to hatching, there is usually an increase in the rate of oxygen consumption above the fitted exponential line (Figures 6.1, 6.2). It is not unreasonable to assume that the skeletal muscles of the embryo would benefit from limbering up exercises prior to hatching. This (variable) prehatching surge in oxygen consumption is evidently preparation for the hatching process and is related at least partly to the increase in muscular activity around this time.

Warburg manometry experiments with a twelve hour photoperiod were run continuously for up to two weeks, with regular observations being made both day and night in an attempt to determine whether the diurnal/ nocturnal rhythm can be initiated prior to hatching, as suggested for snake embryos (Dmi'el, 1969). The average nocturnal oxygen consumption rates were the same as the diurnal rates
($P = 0.271$, $N = 36$ cycles of observations). It is possible that a diurnal rhythm may develop just prior to hatching, as the presence of such a rhythm would be required immediately after hatching for normal nocturnal behaviour.

6.4.7 Respirometry and Incubation Temperature

The Q_{10} value of 2.27 for oxygen consumption during incubation of *U. milii* eggs at 25 °C and 30 °C compares with values for two small sphaerodactyline geckos of 1.95 - 6.08, the highest value was found at temperatures of 20 – 24 °C and the lowest value at 24 – 26 °C (Snyder, 1979). The comparable value at the low end of the published range for geckos is as expected for a larger lizard and suggests that there is nothing remarkably different in the aerobic metabolism of *U. milii* eggs.

The energetic requirements of avian embryonic development can be divided into two components, a growth component and a maintenance component (Vleck and Vleck, 1987). Thus there is an increased energy requirement (in kJg^{-1}) if duration of incubation is increased. The total amounts of oxygen used by the developing embryos at 25 °C and at 30 °C also demonstrated an increased energy requirement for development at the lower temperature by *N. levis* (1 %) and *U. milii* (6 %). However, the increase in *N. levis* may have been reduced by egg retention.

The *Nephrurus* embryos would be expected to utilise more oxygen than *U. milii* embryos because they are larger and more tissue is active; however, because two of the *Nephrurus* clutches of eggs were of abnormally short incubation duration (at 30°C) (possibly due to maternal egg retention), the mean incubation period was markedly reduced and consequently the calculated oxygen consumption was also reduced. Recalculation of oxygen consumption backwards from day of hatching, assuming that the fertilisation date was the same as in other clutches, showed that there was no significant difference in dry mass-specific oxygen consumption between *Nephrurus* and *Underwoodisaurus* embryos. The effects of egg retention also make it difficult to compare any possible difference in energetic requirements of arid and mesic species.

Although most lizards lay eggs with an embryonic developmental stage of about 30 (Dufaure and Hubert, 1961), the increase in rate of oxygen consumption after this stage remains low until approximately 40 % of incubation duration is completed (Thompson and Stewart, 1997; Thompson and Russell, 1999a, 1999b). If this is the case with *Nephrurus*

and *Underwoodisaurus*, then egg retention will not make a large difference in energy consumption; however, the amount is still unverifiable.

6.4.8 Conclusions

Nephrurus levis and *Underwoodisaurus milii* eggs both contain more water than chickens' eggs at laying, and 31 - 33 % additional water is absorbed during incubation, probably at least partly as protection against desiccation. The lack of a selective advantage for high-density lipid energy (as in avian eggs) may contribute towards a lower dry mass proportion of lipids in geckos. Also, the selective advantage of larger eggs and offspring in geckos may contribute towards the increased proportion of proteins in gecko eggs. The near-exponential increase in oxygen consumption during embryonic development suggests no physiological or biochemical inhibition to the energy consumption pattern as occurs in some other reptiles (Thompson, 1989; Thompson and Stewart, 1997). The further increase in oxygen consumption near and at hatching correlates well with increased muscular activity at this time. The mass-specific energy cost of embryonic development is greater in *N. levis* than in *U. milii*, and greater at an incubation temperature of 25 °C than at 30 °C, however; the effect of egg retention in *N. levis* on these measurements is not known. The indirect calculation of the respiratory exchange ratio confirms that embryonic metabolism follows a standard pattern, with metabolic energy derivation almost equally divided between proteins and lipids.

Chapter 7

Conclusions

7.1 Introduction & Lifehistory

This study of the reproductive biology of the endemic Australian diplodactyline gecko genera *Nephrurus* and *Underwoodisaurus* was designed around the assumption that they form a monophyletic group consisting of three distinct clades (all *Nephrurus, U. milii, U. sphyrurus*) (Bauer, 1990b): A derived *Nephrurus* group (*N. deleani, N. levis, N. laevissimus, N. stellatus, N. vertebralis*), and a primitive *Nephrurus* group (*N. amyae, N. asper, N. sheai, N. wheeleri*) are also recognised. These phylogenetic relationships are at least partially supported by the morphological and behavioural aspects of this study.

All *Nephrurus* and *Underwoodisaurus* geckos were confirmed as being moderate to large compared with other Australian geckos. The longevity of *Nephrurus* and *Underwoodisaurus* species was found to be high (16+ years in *Underwoodisaurus* and 6+ years in *Nephrurus*). All species are nocturnal and terrestrial, and generally lay two eggs per clutch in mid to late summer, but with a longer laying season in northern species.

Male: male combat *e.g.* related to territoriality, is unknown in *Nephrurus* species. Such activity probably, does not convey a selective advantage for species with low population densities, hence reduced (currently probably absent) selection for large body size or head size in males. The sparsely distributed *U. sphyrurus* also has no male: male combat. *U. milii* is the only species in the study group known to demonstrate male: male combat and territoriality, possibly because it is often abundant in the areas where it lives (unlike all the other species in the study group).

Nephrurus and *Underwoodisaurus* species are all nocturnal and live in burrows, which means they can escape extremes of temperature. At least some *Nephrurus* species back-fill the entrance to their burrows, thus maintaining burrow humidity and reducing the risk of predation and flash flooding. Predation may also be reduced in all except the *N. asper* group by using tail decoy/baiting behaviour. The low level of natural tail autotomy (as demonstrated by tail regrowth found in preserved specimens) in those species with caudal

autotomy, especially in species with high longevity, suggests that predation pressure may be low. Any such behavioural characteristic that increases individual survival will contribute to the species survival.

7.2 Distributions

The ancestral carphodactyline condition, as exemplified in *Carphodactylus laevis*, was probably mesic terrestrial (Greer, 1989; Bauer, 1990b; Cogger, 2000). However, current carphodactylines include taxa such as *Nephrurus* that are highly adapted to the arid environments of central and northern Australia. In contrast the genus *Underwoodisaurus* is largely mesic and southerly in distribution. The combined distributions of the *Nephrurus* and *Underwoodisaurus* cover almost all of continental Australia, but most areas support only one species.

The finding that diplodactyline species, with their desiccation sensitive eggs, reach their highest species density in arid regions suggests that many species have developed successful adaptations to an arid environment. The finding of higher species densities of diplodactylines compared to gekkonines over most of Australia is probably because of the more recent arrival of gekkonines (Kluge, 1967a) (that have desiccation resistant eggs) rather than the better arid adaptation of diplodactylines. The fact that other squamates laying flexible-shelled eggs have high species diversity in arid Australia suggests that the increased speciation may actually be driven by desertification.

7.3 Morphology

Observational evidence suggests that the highly distinctive caudal knobs of *Nephrurus* species tails may have a pheromonal reproductive function. The size of the caudal knob was not correlated with size of tail and did not significantly increase in size at maturity. The caudal knob (whether large or small or whether attached to a moderate sized or very small tail) was found to be actively used in a range of situations (including courting and mating), suggesting the activity may be part of a generalised stress response.

The similar size and morphology, allopatric distributions and similar behaviours of the primitive *N. asper* group, including the spinose scalation, the same phalangeal formula and

lack of a caudal autotomy plane (present in *N. wheeleri*) supports the possible monophyly of this group within *Nephrurus*.

The confirmation of an exceptional female-biased sexual size dimorphism in all *Nephrurus* species was linked to large egg size in arid species. The lack of a significant sexual size dimorphism in *U. milii* was linked to a mesic habitat (where smaller eggs have a better chance of survival) and territorial behaviour in the male.

The well-differentiated and distinctive preano-inguinofemoral tubercles that were found in the adult males of the derived *Nephrurus* group (except *N. vertebralis*) which are presumed to have a reproductive function (possibly related to mating receptivity of the female), also give support to the above phylogenetic relationships of the two *Nephrurus* clades.

The finding of increased relative transtubercular measurements in the males in 10 out of 11 study group species suggests that the cloacal tubercles are sexually important, but the precise function remains unconfirmed.

7.4 Reproduction

Diplodactyline nest sites were found in moist substrates (no *Nephrurus* or *Underwoodisaurus* sites were found in the wild) in deep crevices or at some depth below the surface (never superficial, as often occurs in gekkonine species), thus increasing protection against aridity. The *Nephrurus* and *Underwoodisaurus* nest site selection strategy in captivity of laying eggs deeply in burrows adjacent to or beneath large rocks, logs or vegetation will increase arid survival of eggs, at least in some cases. Such sites can be expected to experience lesser extremes of both temperature and water potential.

Nephrurus and *Underwoodisaurus* species were found to have seasonal iteroparity in captivity and probably also in the wild, thus giving opportunity, particularly in favourable seasons, for an increase in fecundity. *Nephrurus laevissimus and N. levis* (and to a lesser extent *U. milii*) have a prolonged population breeding season in the wild (probably but not necessarily, with seasonal iteroparity). Irregular iteroparity may be important in sustaining populations, particularly in areas and times of adverse and variable meteorological, edaphic and ecological conditions.

The presence of sperm storage in *Nephrurus* females is probably advantageous for non-social species with low population density, and where environmental conditions may often be unsuitable for egg laying. However more data are required for confirmation of the frequency of sperm storage in *Nephrurus*.

The exceptionally large female-biased sexual size dimorphism in *Nephrurus* species is a strong indicator that there is a selective pressure for increased size in females only. Adult *Nephrurus* (and *U. milii*) males are larger than the adult males and females of the majority of other Australian diplodactyline geckos suggesting, although not conclusively, that the female-biased sexual size dimorphism is not generated by selection for smaller males. The fact that the size dimorphism is also greater in arid than in mesic diplodactyline species, also strongly supports the hypothesis that there is a selective advantage for increased female size in arid species.

The reason why there is no female-biased sexual size dimorphism in *U. milii* is probably because, being mesic, the species has less selective pressure for the females to grow larger in order to produce larger eggs. Also, this species demonstrates male: male combat and territoriality, which is commonly associated with increased size in males. If the primitive state is larger female size then there may have been slightly greater selection pressure on males (compared to females) for increased size (the fact that *U. milii* is a moderately large species compared to most diplodactyline species suggests that selection has not been towards decrease in size).

There was minimal evidence of senescence or atrophy of gonads in the largest and presumably oldest specimens, indicating a long reproductive life so that populations can survive several years of low or possibly zero recruitment. Reproduction was found to begin at a larger size (presumably older,) in *Nephrurus* females than in males, which allows females to reach adequate size to produce the large eggs needed for increased arid resistance. Juvenile *N. levis* and *U. milii* males and females in captivity were found to grow at almost identical rates under similar conditions. The smaller size and earlier sexual maturity in males brings a larger cohort into earlier reproduction, thus increasing the chance of mating and of multiple matings for a given female, which may be a significant advantage in sustaining a population that often is low-density (Chapter 2). The near parity of adult sex

ratios in 10 out of the 11 study species suggests that there is no differential predation pressure against males (or females) that might counter this conclusion.

Nephrurus and *Underwoodisaurus* species have a predominant clutch size of two eggs, but under certain (possibly adverse) conditions may reduce the clutch size to one.

7.5 Eggs

The primitive mesic terrestrial carphodactyline gecko *Carphodactylus laevis* produces relatively small eggs; the presumed ancestral carphodactyline state. However, diplodactyline (and arid-adapted eublepharine) eggs were found to be relatively larger than gekkonine eggs. The largest gecko species (producing the relatively and absolutely largest eggs) were diplodactylines and the smallest gecko species (producing the relatively and absolutely smallest eggs) were sphaerodactylines (small gekkonines also produce relatively and absolutely smaller eggs than diplodactylines). Also, the absolute minimum size of gekkonine eggs is much smaller than the smallest diplodactyline eggs, and the largest diplodactyline eggs are much larger than the largest gekkonine eggs, as would be expected if there were no selective pressure on arid gekkonines to produce larger eggs. These data correlate with the differences in eggshell type. Rigid-shelled (endohydric) (sphaerodactyline and gekkonine) eggs were found to be highly resistant to desiccation, whereas flexible-shelled (ectohydric) (diplodactyline and eublepharine) eggs were desiccation sensitive. By reason of their larger size and increased water content, the ectohydric eggs of *Nephrurus* species have increased resistance to desiccation because of their increased volume to surface area ratio. Thus, there is a selective advantage for species able to produce larger eggs in arid regions. Another characteristic of diplodactyline (including *Nephrurus*) species' eggs is that they can absorb moisture (partly by reason of their ellipsoidal shape and partly by reason of their flexible shells) from the substrate, thus increasing their water reservoir against possible desiccation stress.

The relative clutch mass is significantly greater in arid than mesic diplodactyline species of similar SVL *i.e.* the selective pressure is primarily to produce larger eggs and therefore larger females to produce them. The increase in RCM correlates well with the idea that females in arid environments have experienced selective pressure to increase their egg and hatchling sizes.

The eggs of diplodactyline geckos from arid environments are more desiccation resistant than the eggs of those from mesic environments, by reason of their increased size and volume to surface area ratio (Chapter 5), and possibly by reason of both increased shell calcium carbonate and increased thickness of the shell membrane (not confirmed in this study).

7.6 Respirometry and Energetics

The pattern of oxygen consumption during incubation is approximately exponential, in line with embryonic development, and similar to the pattern found in many other lizards. The embryonic mass-specific energy consumption, is also similar to that of other lizard species, as determined from respirometry and egg analysis, and involves both maintenance and development components.

The total amounts and proportions of water, lipid and protein in the eggs are variable, but similar to concentrations in other lizard eggs. The embryonic metabolic energy derivation is almost equally divided between proteins and lipids. The egg lipid provides sufficient energy to allow rapid embryonic development, which is probably needed for the large hatchling to become established in its environment prior to onset of winter (particularly in non-tropical species).

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Appendix 1

1.1 Morphometric Characters Analysed

Most of the specimens measured were preserved museum specimens but wherever possible live specimens were also measured (as specified in Appendix 2).

The characters examined and their abbreviations and related comments follow:

1. Snout-Vent Length (SVL). This was measured from mid-rostral to anterior margin of cloacal aperture.
2. Tail length (from vent to tail tip). Whether the tail was original, missing or regrown was also recorded. Total Length (TL) was derived by addition of SVL and tail length.
3. Head width (HW). This was measured at the widest point of the head excluding any enlarged tubercles. In most cases this was in the area between the lateral extremities of the postfrontal bones and the lateral extremities of the quadrate bones.
4. Head height (HH). This was measured just behind the eyes at the estimated suture of the frontal and parietal bones.
5. Head length (HL). This was measured as the straight-line distance from the anterior inferior tympanic opening to the centre of the rostral scale.
6. Ear height (EH). This was measured across the maximum diameter of the opening, which is usually vertically elliptical and tends to slope slightly downwards and forwards. In *Nephrurus* geckos the tympanic closure muscle is oriented vertically along the posterior and inferior margins of the auditory meatus and closes the opening to form a near vertical slit, ie. this is not a true sphincter muscle. Consequently the state of contraction of this muscle has only a minimal effect on vertical diameter (personal observation).
7. Transverse eye diameter (of exposed portion) (ED). It is possible that factors such as preservation technique, desiccation, starvation and constriction of the *depressor ocularis* muscle may affect this measurement thus reducing the validity of the measurement.
8. Tail width (TW). The maximum transverse value, excluding enlarged tubercles, this measurement is variable within species particularly among those species with larger tails.
9. Tail height (TH). The maximum height of tail, excluding tubercles, and measured just

posterior to any post-anal fold (if present). An estimate of tail volume was derived from the tail measurements using the formula for the volume of an elliptical cone $V=\pi r_1 * r_2 l/3$. Where V = volume (mm3), r_1 and r_2 = half the TW and half the TH in mm and l = tail length in mm.

10. Peduncle (Ped.), or peduncle length (PL). This was measured as the length of the cylindrical, non-depressed or non-expanded, pre-knob section of tail, or equivalent portion of tail in *Underwoodisaurus* tails, measured from point of inflection when viewed from above.
11. Tail knob width (KW). This was measured as the widest part of the terminal tail knob in *Nephrurus* species.
12. Tail knob height (KH). This was measured as the maximum height value for the tail knob.
13. Tail knob length (KL). This was measured from the last of the annular peduncle creases to the tip of tail knob. From these measurements a value for the knob volume could be obtained using the formula for a laterally squashed prolate spheroid, *i.e.*, with three different perpendicular ellipsoidal planes. $V = 4*\pi*r_1*r_2*r_3/3$. Where V = volume in mm^3, and r_1, r_2, and r_3 are half the values of KW, KH and KL in mm.
14. Tail knob scales (TKSc). This number was counted transversely around the widest part of the caudal knob.
15. Transtubercular pelvic width (TtW). This was measured across the pelvis from tip to tip of post-cloacal (or spur) tubercles.
16. Transiliac pelvic width TiW). This was measured across the pelvis immediately above insertion of femora.
17. Width of mental scale (MW). This was measured as the maximum straight-line width across this scale.
18. Height of mental scale (MH). This was measured as the maximum straight-line height (or longitudinal diameter) of this scale.
19. Width of rostral scale (RW). This was measured as the maximum straight-line width across this scale.
20. Height of rostral (RH). This was measured as the maximum straight-line height of this scale. The total areas (RMA) of these scales were calculated by addition of the products from each scale. Although sometimes irregular and never flat/rectangular these

measurements were believed to be adequate for interspecific comparative purposes.

21. Forelimb length (FL). Limb length was measured from intersection of limb with torso to the tip of the longest extended digit while the limb was held horizontal and perpendicular to the long axis of body. Where the point of intersection was indistinct the proximal locus was determined as the point at which the axillary skin subtended an angle of 45° to the long axis of the limb.
22. Leg length (HL). Was measured as above.
23. Mid-body width (MBW). This was measured as the maximum transverse width of the torso excluding height of any enlarged conical tubercles.
24. Number of supralabial scales (SL). The labials were counted on both sides and included all enlarged, shiny, labial-margin-modified scales.
25. Number of infralabial scales (IL). These were measured as above.
26. Number of nasolabial scales (NL). This was the minimum number of contiguous scales between nostril and labial/rostral scales and was counted on both sides.
27. Number of interorbital scales (IO). This was counted as the minimum number of contiguous scales between the medial supraocular creases and including the usually enlarged medial parasulcal tubercles. In *Underwoodisaurus* species where the sulci and enlarged tubercles are absent or indistinct the count was made from an estimated equivalent position related to the orbital margin of the skull.
28. Number of internasal scales (IN). This was counted as the minimum number of contiguous scales between the nares across top of snout.
29. Number of post-rostral scales (PR). This was counted as the number of scales contacting the upper margin of the rostral scale. In *Underwoodisaurus* species this includes the enlarged anterior nasal scales (called supranasals by some authors).
30. Number of post-mental scales (PM). This was counted as the number of scales contacting the lower margin of the mental scale.
31. Number of naso-ocular scales (NO). This was counted as the minimum number of contiguous scales between the nostril and the preocular crease, counted on one side only.
32. Number of perinasal scales (PN). This was counted as the number of contiguous scales contacting the margin of the annular naris scale, counted on one side only.
33. Number of rosette scales (RS). This was counted as the modal value of 10 or more counts of the scales forming a rosette or annulus around the enlarged dorsal tubercles.

34. Number of inter-rosette scales (Inter-R). This was expressed as a range of numbers of scales found between rosettes of enlarged dorsal tubercles of major rosettes ie. excluding the rosette scales, in the mid-dorsal region (no STD or t-Tests done on these ranges).
35. Number of mid-body scales (MBS). This was counted as the number of contiguous granular scales around mid-body avoiding the enlarged tubercles and enlarged rosette scales where present.
36. Number of dorsal scales from rostral to autotomy plane (DSRA). This was counted as the number of contiguous dorsal scales along the mid-vertebral line avoiding the enlarged tubercles and enlarged rosette scales wherever possible. In the case of the *N. asper* group which have no autotomy plane the count was made to the basal tail sulcus.
37. Number of ventral scales from mental scale to vent (VSMV). This was counted as the number of ventral scales along the mid-ventral line but avoiding any enlarged umbilical scales found in some *Nephrurus* and *Underwoodisaurus* specimens.
38. Number of palpebral margin scales (PMS). This was counted as the number of contiguous scales along the ridge between posterior-most (usually conical to spinose) and anterior-most, excluding scales in vertical series at either extremity. Counted on one side only.
39. Minimum number of transpalpebral scales (TPS). This number was counted as the number of contiguous scales in a transverse series across the eyelid margin. The scales were counted from the trough of the lateral supraocular sulcus to the margin at position of maximum width and counted on one side only.
40. Testes (T) dimensions. Museum specimens were dissected (if not already dissected) and the length, width and depth of each testis was measured *in situ*. A very small percentage were damaged eg. by desiccation, trauma or disease and could not be measured. Testes removed for histological preparation were measured after the orchidectomy. These measurements enabled the determination of accurate values for testicular volume using the formula for a squashed prolate spheroid *i.e.* with three different perpendicular ellipsoidal planes. $V = 4*\pi*r_1*r_2*r_3/3$. Where V = volume in mm^3, and r_1, r_2, and r_3 are half the values of testis width, length and height in mm.
41. The cube root of the combined testicular volumes was then used as an index for comparison with snout-vent length (SVL).
42. Ovarian follicle (F) dimensions. In most specimens the diameters only were measured

of the largest three or four follicles in each ovary on left (Lt.) or right (Rt.) side and/or eggs in oviducts measured in mm. They were numbered in decreasing order of diameters (FLt1, FLt2, FLt3... etc. on left and FRt1, FRt2, FRt3...etc on right). Thus FLtD1 is the diameter of the largest follicle in the left ovary etc.

43. The fat body was arbitrarily classified as being absent (1), very small (2), small (3), moderate (4), large (5) or very large (6). The fat body is rather flimsy, floppy and friable and difficult to measure accurately. Also because some past dissections had damaged or removed this organ (especially if not well preserved) some of the measurements may be underestimates of original dimensions. Although not entirely satisfactory these semiquantative estimations are believed to be adequate for the comparative purposes required.
44. The number of subdigital lamellae (SDL). The lamellae were counted on each digit (for *Underwoodisaurus* species). Digits were numbered in the standard manner 1-5 (anterior to posterior and manus defined prior to pes). In certain cases the number of lamellae was limited in extent to the base of the interdigital webbing because of the presence of enlarged lamellae on the volar surfaces or because of the sometimes gradual intergradation of SDLs and volar scales and also on occasion a divided lamella near the base of the digit which tended to extend or confuse the apparent number of SDLs. The apical lamella only is divided longitudinally in *U. milii* (as in most Diplodactylinae) but counted as one. Because the digital scales of *Nephrurus* species are multicarinate and deeply grooved it is not possible to determine if scales are divided or to count scales around the digit. Except for the most distal digital scales, which are in about 5-10 annular arrays around the toes, the scales are arranged irregularly and therefore cannot be counted for comparison with *Underwoodisaurus* species.

STD = standard deviation.

N = number of observations.

Lt (or L) and Rt (or R) = left side and right side respectively.

Vol. (or V) = volume.

Sc. = Scale(s).

(m+f) = combined males and females.

L = Length.

NA = not applicable.

Date = date of collection of museum specimens. The dates here have been decimalised and where (in a few cases) only the month is given 0.5 is added to give the nearest value on average to the real value. Midwinter is defined here as end of June so that start of the season of activity is defined by the first date of collection after July the first, and ends on the last date prior to June thirtieth. In the case of species confined to the tropical region the period of activity may encompass all months of the year. It should be borne in mind however that in some (very few) cases it is possible that collection dates may not be accurate for a variety of reasons also a few specimens may have been collected from their winter retreats. In the case of living specimens the 'Date' means date of observation and recording of data and not necessarily the date of collection (in the case of live specimens this is not used for determination of activity season). Unless indicated otherwise the data presented here relates to the specimens personally examined. In species where large numbers of museum specimens are held the number examined may not include all available specimens of that species.

The Students t-Test results are two-tailed homoscedastic (equal variance) for all meristic characters which are presumed (and observed in several species) to remain constant during ontogenetic development. The t-Test results for all other mensural characteristics are for one-tailed homoscedastic tests because it is presumed that these factors do not change until sexual maturity is approached and that young juveniles of both sexes will be of very similar proportions in respect to these characters. Unless otherwise indicated the statistics have been applied to all the material available (including juveniles) in the given category.

The incidence of overt gonadal pathology was extremely low across all species studied at <0.5% (N = 405 x 2), although two cases of *Cryptosporidium* infection of the ovary were discovered by histology. This low level of disease suggests that results are unlikely to have been significantly affected by pathology.

1.2 Tables of Morphometric Data

Table A1.1 Morphometric Characters For *N. amyae*.
For unspecified abbreviations see previous line(s) or list above.

	MALES				FEMALES				
CHARACTER	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Collection Dates		10.8-5.3		9		10.4-3.5		6	
SVL	88.0	50.5-110	20.4	9	114	51.3-137	24.3	10	.051
Tail Length	17.6	23.9-17.6	3.08	8	21.8	11-27.4	4.84	10	.071
Total Length	104	53.3-134	25.6	8	135	62.3-163	28.4	10	.091
Tail as % SVL	19.1	16.5-21.8	2.03	8	19.4	16.4-24.8	2.47	10	.303
Head Width	22.5	13.8-29.8	5.87	9	30.6	13.2-36.0	6.61	10	.043
HW as % SVL	26.4	24.3-27.9	1.26	9	26.9	24.5-29.1	1.36	10	.175
Head Height	11.8	8.00-15.0	2.88	6	15.8	8.10-19.2	3.72	7	.079
HH as % SVL	14.4	13.6-15.8	.813	6	14.6	12.9-15.8	.967	7	.238
Head Length	23.7	16.0-30.2	5.72	6	29.7	15.7-35.2	6.40	7	.139
HL as % SVL	29.0	27.5-31.7	1.45	6	27.7	25.8-30.6	1.77	7	.126
Tail Width	6.55	4.52-9.80	1.91	6	8.02	3.27-11.7	2.35	10	.172
TW as % SVL	7.12	5.26-9.16	1.31	6	7.03	5.54-9.14	1.26	10	.483
Tail Height	4.06	2.42-5.60	1.23	5	5.63	2.50-8.70	1.68	8	.086
TH as % SVL	4.61	3.45-5.10	.672	5	5.05	4.46-6.80	.746	8	.072
Tail Volume	124	42.7-262	87.0	5	274	23.5-666	124	8	.095
3/ TV	4.72	3.50-6.40	1.18	5	6.15	2.87-8.73	1.63	8	.073
3/ TV as % SVL	27.0	23.5-28.9	2.10	5	28.7	24.6-34.9	3.43	8	.117
Peduncle	2.75	2.09-3.00	.379	5	3.60	2.23-4.50	2.75	7	.106
Ped. as % SVL	3.17	2.72-4.18	.602	5	3.39	2.42-4.35	.662	7	.246
Ear Height	4.86	2.50-6.60	1.66	7	6.04	2.36-8.30	4.86	7	.215
EH as % SVL	5.55	4.91-6.41	.567	7	5.47	4.60-6.38	5.55	7	.411
Eye Diameter	5.57	3.90-7.00	1.24	6	6.67	4.10-8.20	5.57	6	.216
ED as % SVL	6.87	5.83-7.72	.623	6	6.34	5.45-7.99	.886	6	.166
Post Rostrals	10.1	8-12	1.27	9	8.89	7-10	1.05	9	.052
Post Mentals	9.00	7-12	1.66	9	9.11	8-11	1.27	9	.668
Transtubercular W	9.31	4.20-15.2	3.55	6	11.8	4.28-15.1	4.53	7	.227
T tW as % SVL	10.7	8.28-13.8	2.55	6	10.7	8.34-12.9	1.53	7	.045
Transiliac W	9.65	5.40-13.7	3.22	6	13.3	5.50-16.8	3.67	7	.207
TiW as % SVL	11.6	10.7-12.5	.71	6	12.1	10.7-12.9	.68	7	.222
SpurTubercles	22.5	16-32	7.51	4	23.0	20-26	4.24	3	.407
Body Width	22.9	12.1-31.0	6.27	8	28.3	11.8-38.4	8.16	10	.145
BW as % SVL	25.9	23.0-29.0	2.54	8	25.23	19.1-32.0	4.42	10	.387
Fat Body*	3	2-4	1.00	5	4	3-5	0.58	7	.026
Lt Testis Length	4.97	1.77-6.32	1.87	5					
Lt T Width	3.79	1.10-6.00	1.76	5					
Lt T Depth	2.37	0.77-4.50	1.42	5					
Lt T Volume	33.2	0.79-89.1	33.5	5					
Rt T Length	4.85	1.85-6.1	1.81	5					
Rt T Width	3.91	1.07-5.8	1.82	5					
Rt T Depth	1.99	0.67-3.60	1.14	5					
Rt T Vol.	26.9	0.69-66.7	24.7	5					
Lt+Rt Tvol.	60.0	1.48-156	58.2	5					
Lt Follicle 1 Diameter					5.61	1.95-13.4	3.99	6	
LF2D					2.85	0.45-3.74	1.22	6	
LF3D					2.16	0.21-2.72	0.977	6	
Rt Follicle 1 Diameter					4.69	1.15-14.0	4.31	7	
R F 2					2.63	.32-3.55	1.19	6	

Table A1.1 (Continued).

N. AMYAE	MALES				FEMALES				
CHARACTER	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
R F 3					2.52	2.24-2.81	0.248	5	
LFD1+RFD1as% SVL					9.95	5.65-23.48	6.80	6	
Rostral Width	3.69	2.92-4.71	0.83	6	4.28	3.41-5.11	0.552	5	.216
RW as % SVL	4.57	4.16-5.84	0.64	6	4.04	3.41-5.11	0.55	5	.138
R Height	1.26	0.97-1.72	0.30	6	1.55	0.90-2.07	0.36	5	.436
RH as % SVL	1.56	1.28-2.00	.251	6	1.49	1.29-1.75	.223	5	.335
Mental Width	2.56	1.92-3.09	0.48	6	3.14	2.40-3.88	0.56	5	.059
MW as % SVL	3.19	2.81-3.80	0.40	6	3.03	2.33-4.68	0.78	5	.464
M Height	0.808	0.65-1.11	0.16	6	1.04	0.74-1.31	0.21	5	.278
MH as % SVL	1.017	.699-1.29	.199	6	1.06	.794-1.44	.321	5	.405
R + M Area	16.0	13.4-20.4	2.70	6	9.28	7.96-11.9	1.68	5	.339
/(RMA)	3.99	3.66-4.51	.332	6	3.04	2.82-3.45	.268	5	<.0001
/(RMA) as %SVL	5.27	3.55-8.63	2.03	6	3.03	2.22-5.53	1.14	5	.025
Internasals	18.6	17-21	1.51	9	17.4	16-19	0.98	7	.058
Interorbitals	7.44	7-8	0.53	9	7.44	6-10	1.13	9	.720
Nasolabials	4.63	4-5	0.52	16	4.73	3-7	1.09	14	.484
Perinasals	22.3	21-24	1.53	3	20.3	20-21	0.577	3	.096
Nasooculars	34.5	30-37	2.88	6	35.2	32-40	3.06	6	.766
Supralabials	14.2	11-16	1.58	11	15.1	13-17	1.65	18	.248
Infralabials	15.2	14-17	1.08	12	14.4	11-17	1.73	15	.674
Transpalpebral	7.67	7-8	0.52	6	8.14	8-9	0.38	7	.072
Palpebral Edge	30.8	28-34	2.64	6	29.7	28-32	1.50	4	.476
Midbody Scales	279	258-298	16.7	4	287	281-293	6.93	4	.276
Rostral-Pelvis Sc	397	346-439	41.5	4	377	350-406	28.8	4	.478
Mental-Vent Sc	337	319-374	26.0	4	358	345-370	10.2	8	.184
Rosette Scales*	6	6	0	6	6	6	0	8	1.00
Inter-R Scales		2-10		3		0-10		2	NA
Knob Width	3.69	2.80-4.30	0.53	6	4.60	2.86-5.70	0.76	10	.035
Knob Height	2.93	2.21-3.30	0.39	6	3.51	2.21-4.10	0.56	9	.065
Knob Length	3.12	2.22-3.60	0.52	6	3.91	2.22-5.00	0.76	10	.062
Knob Volume	18.5	7.19-24.3	6.64	6	34.7	7.35-61.2	14.2	10	.032
3/KV	2.60	1.93-2.90	0.37	6	3.08	1.94-3.94	.616	10	.056
3/KV as %SVL	2.87	2.52-3.48	.335	6	2.99	2.32-3.96	.549	10	.329
Isthmus Width	1.18	0.89-1.45	0.21	6	1.41	0.99-1.76	0.23	9	.028
I W as % SVL	1.30	0.94-1.66	0.23	6	1.32	0.99-1.93	0.32	9	.252
Caudal Annuli	10.1	9-11	0.74	5	10.6	9.50-11.5	0.79	8	.292
Knob Scales	39.8	37-43	2.39	5	38.3	35-42	3.01	7	.308
Forelimb Length	37.1	22.9-46	8.38	9	44.9	21.9-55.0	9.74	10	.132
FL as % SVL	42.3	39.9-45.4	2.26	9	40.3	38.1-42.7	1.43	10	.016
Hindlimb Length	43.0	25.7-54.3	9.75	9	53.0	24.2-66.0	12.1	10	.113
HL as % SVL	48.9	46.7-50.9	1.51	9	47.4	44.3-48.8	1.31	10	.009
Subdigital Lamellae	NA								
Latitudinal Range (m+f)		12.7-24.0°S							
Longitude Range (m+f)		132.6-136.9°E							

Table A1.2 Morphometric Data Analysis For *N. asper*

	MALES				FEMALES				
CHARACTER	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Collection Dates		11.1-4.5		11		10.5-6.0		12	
SVL	77.5	35.2-104	15.7	29	89.4	45.5-118	22.9	30	.116
Tail Length	17.6	7.40-23.5	3.93	29	17.5	11-26	4.21	29	.247
Total Length	95.1	42.6-128	19.4	29	106	57.5-140	26.8	29	.207
Tail as % SVL	22.7	16.5-24.7	1.63	29	20.1	16.0-26.4	3.15	29	0.0025
Head Width	22.7	9.54-30.3	4.94	26	25	12.5-31.7	5.52	26	.339
HW as % SVL	29.0	24.8-33.3	2.05	26	27.1	24.7-30.4	1.37	26	.00014
Head Height	10.1	5.60-14.2	3.17	7	12.6	7.67-16.8	.537	7	.096
HH as % SVL	14.6	12.0-16.1	1.66	7	16.3	16.2-16.7	.175	7	.011
Head Length	19.9	11.1-25.8	5.62	7	24.1	13.4-30.3	7.08	7	.122
HL as % SVL	28.9	26.1-31.5	2.17	7	28.9	25.8-30.0	1.53	7	.079
Tail Width	6.13	2.9-8.5	1.40	19	6.20	4.3-8.00	1.24	19	.192
TW as % SVL	8.69	5.68-10.3	1.53	13	7.28	5.50-10.0	1.44	19	.0059
Tail Height	3.83	2.28-5.8	1.15	7	2.89	2.87-2.91	.028	7	.418
TH as % SVL	5.46	3.90-6.48	1.04	7	5.92	5.52-6.33	.571	7	.141
Tail Volume	34.4	29.3-38.4	3.08	7	25.1	16.0-42.4	7.41	7	.365
$3\sqrt{}$ TV	11.6	6.25-16.3	3.23	7	12.4	8.64-14.7	2.83	7	.461
$3\sqrt{}$ TV as % SVL	16.4	13.6-17.9	2.06	7	15.2	12.8-18.8	2.03	7	.117
Peduncle	3.23	1.70-4.33	1.04	7	4.01	2.71-4.80	.851	7	.075
Peduncle % SVL	4.65	4.19-5.01	.300	7	4.97	3.91-6.04	.756	7	.330
Ear Height	3.37	1.68-5.22	1.34	7	2.69	2.41-2.97	.396	5	.225
EH as % SVL	5.07	3.93-6.04	.761	7	5.48	5.24-5.71	.334	5	.352
Eye Diameter	4.74	2.69-6.26	1.39	7	4.09	3.91-4.27	.255	5	.177
ED as % SVL	7.31	6.29-7.93	.592	7	8.36	8.21-8.50	.204	5	.299
Post Rostrals	13.0	11-16	1.8	16	12	9-15	1.9	11	.382
Post Mentals	9.60	8-12	1.3	15	10	7-14	2.3	11	.450
Transtubercular W	10.0	2.72-13.9	3.74	12	8.83	5.00-14.8	3.15	8	.019
T tW as % SVL	12.7	5.52-16.1	3.13	12	10.2	8.60-12.5	1.18	8	<.0001
Transiliac W	7.66	3.47-11.2	3.05	6	5.64	4.88-6.40	1.08	5	.019
TiW as % SVL	11.2	9.33-12.3	1.29	6	11.5	10.6-12.3	1.20	5	.154
Spur Tubercles	14.3	4-25	6.61	9	13.5	12-15	2.12	7	.931
Body Width	16.7	6.43-23.7	5.35	7	11.7	10.8-12.6	1.27	9	.316
BW as % SVL	26.7	18.3-33.2	4.41	7	24.1	20.8-27.4	4.68	9	.102
Fat Body*	4.5	4-5	.577	4	3.5	2-5	2.12	7	
Lt Testis Length	5.88	1.12-10.9	2.17	15					
L T Width	4.18	.440-5.7	1.40	15					
L T Depth	2.89	.370-4.70	1.04	15					
L T Volume	45.5	.096-108	30.4	15					
Rt Testis Length	5.79	1.10-11.3	2.26	14					
R T Width	4.06	.420-6.10	1.33	14					
R T Depth	2.83	0.35-4.30	1.01	14					
R T Volume	43.4	.085-122	33.0	14					
Lt+Rt Tvol	91.8	.180-231	61.8	14					
Lt Follicle 1					9.44	0.50-28.9	11.4	10	
Rt Follicle 1					11.5	0.60-27.5	11.9	10	
LFD1+RFD1as%SVL					11.2	1.15-26.7	11.1	10	

Table A1.2 (Continued)

N. ASPER									
CHARACTER	MALES				FEMALES				*T*-TEST
Rostral Width	3.31	2.20-4.61	.898	6	2.66	2.40-2.92	.368	9	.357
RW as % SVL	5.09	4.63-6.25	.592	6	5.12	5.22-5.62	.281	9	.151
R Height	1.05	0.52-1.56	.380	6	0.88	0.81-0.95	.099	9	.137
RH as % SVL	1.55	1.47-1.71	.090	6	1.56	1.31-1.83	.233	9	.459
Mental Width	2.10	1.60-2.85	.870	6	2.00	1.66-2.33	.474	9	.136
MW as % SVL	3.33	2.10-4.55	.792	6	4.05	3.61-4.48	.617	9	.409
M Height	.762	0.44-0.98	.192	6	0.59	0.57-0.61	.028	7	.180
MH as % SVL	1.21	.838-1.90	.396	6	1.08	.881-1.33	.180	7	.218
R+M Area	5.40	2.22-9.99	3.53	6	3.53	2.96-4.10	0.81	7	.113
√(RMA)	2.26	1.49-3.16	.617	6	2.70	1.72-3.41	.659	7	.119
√(RMA) as % SVL	3.46	3.02-4.23	.419	6	3.39	2.95-3.90	.418	7	.118
Internasals	18	16-21	1.69	8	16	14-18	2.83	7	.146
Interorbitals	8.44	7-9	.726	9	9	7-11	2.83	7	.596
Nasolabials	4.86	4-6	.754	14	4.22	2-6	.577	18	.011
Perinasals	21.3	19-24	1.89	7	20	19-21	1.41	7	.902
Nasooculars	29.6	25-32	2.64	7	26.5	26-27	.707	9	.006
Supralabials	14.2	12-16	1.14	20	13.9	12-16	1.02	18	.464
Infralabials	14.4	13-16	.966	10	14	13-15	1.41	9	.808
Transpalpebral	6.5	6-7	.548	6	7.0	7	0	7	.034
Palpebral Edge	26	24-30	2.53	6	26	24-28	2.83	7	.800
Midbody Scales	278	243-300	21.4	5	291	274-307	23.3	5	.880
Rostral-Pelvis Sc	438	392-472	34.3	5	406	393-419	18.4	5	.034
Mental-Vent Sc	344	312-405	158	5	323	319-326	4.95	5	.524
Rosette Scales	7	7	0	3	6.5	6-7		7	NA
Inter-R Scales		2-9		3		3-8		5	NA
Knob Width	4.00	2.4-5.5	0.70	23	4.09	2.8-5.5	.661	22	.342
Knob Height	3.48	2.38-4.7	0.72	15	3.00	2.5-3.9	0.41	12	.0067
Knob Length	3.12	1.91-4.50	.713	15	3.42	2.8-4.5	.497	12	.223
Knob Volume	24.8	5.71-55.4	13.7	15	21.3	11.4-44.1	9.8	12	.094
3√ KV	2.86	1.79-3.81	.557	15	2.72	2.25-3.53	.382	12	.158
3√KV as %SVL	3.86	2.89-5.09	3.40	15	3.41	2.75-4.90	.862	12	.428
Isthmus Width	1.25	0.90-1.84	.288	12	1.18	1.17-1.18	.007	7	.300
I W as % SVL	1.94	1.25-3.13	.548	12	2.41	2.25-2.57	..227	7	.234
Caudal Annuli	9.79	9-11	.756	7	10	10	0	7	.054
Knob Scales	41.9	39-46	2.70	8	40.5	37-44	4.95	8	.880
Forelimb Length	31.1	15.5-39	6.80	17	35.3	19.8-46	2.02	13	.406
FL as % SVL	43.3	39.3-47.8	2.88	17	41.1	37.7-45	2.44	13	.074
Hindlimb Length	36.7	16.1-47.5	8.64	17	39.5	21.5-52.0	12.0	13	.375
HL as % SVL	50.7	45.7-57.3	3.43	17	46.0	41.0-52.0	3.02	13	.00017
Subdigital Lamellae	NA								
Latitudinal Range (m+f)		10.9-25.8°S							
Longitudinal Range (m+f)		119.6-150.5°E							

Table A1.3 Morphometric Analysis For *N. deleani*

	MALES				FEMALES				
CHARACTER	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Collection Dates		10.8-4.8		4		10.8-4.8		3	
SVL	53.5	36.0-67.0	12.4	8	72.1	45.2-89.0	14.3	12	.0039
Tail Length	20.3	12.5-26.1	5.46	7	26.4	17.2-33.0	4.34	12	.0099
Total Length	71.6	51.0-92.0	18.7	8	98.5	62.4-115	18.4	12	.0026
Tail as % SVL	37.6	32.1-41.7	3.12	7	36.9	28.1-41.3	3.64	12	.332
Head Width	14.8	9.50-17.8	2.87	7	17.3	11.9-20.7	2.87	11	.051
HW as % SVL	25.5	22.3-29.8	2.60	7	24.6	22.8-27.4	1.41	11	.192
Head Height	7.28	5.40-9.10	1.85	5	9.25	5.87-10.8	5.87	6	.085
HH as % SVL	14.1	13.9-14.4	.287	5	12.3	10.2-13.0	1.06	6	.014
Head Length	15.4	11.7-18.1	3.32	5	21.2	17.0-23.6	2.60	5	.016
HL as % SVL	30.0	27.9-32.2	2.11	5	28.6	27.1-29.7	1.35	5	.143
Tail Width	5.13	2.40-7.60	2.61	4	4.75	2.60-7.20	1.41	9	.244
TW as % SVL	8.59	6.15-11.3	2.57	4	6.78	5.25-9.33	1.39	9	.340
Tail Height	3.97	2.00-5.30	1.74	4	4.32	2.71-5.10	.831	6	.143
TH as % SVL	6.70	5.13-7.86	1.41	4	5.77	4.73-6.79	.698	6	.321
Tail Volume	235.2	49.0-711	332	4	422	165-540	137	6	.108
3√ TV	6.69	3.66-8.93	2.72	4	7.40	5.49-8.14	.986	6	.286
3√ TV as % SVL	11.4	9.38-13.2	1.94	4	9.97	9.09-11.8	1.02	6	.088
Peduncle	10.3	8.3-13.3	2.67	4	10.0	8.30-13.2	2.16	3	.341
Ped. as % SVL	16.7	12.3-19.9	3.92	4	14.0	11.5-16.5	3.57	3	.244
Ear Height	2.10	1.80-2.27	.258	5	2.88	1.42-3.90	.903	5	.103
EH as % SVL	4.16	3.50-4.62	.581	5	3.80	2.48-4.70	.828	5	.270
Eye Diameter	4.46	3.50-5.49	.997	5	5.58	4.03-6.60	1.07	5	.096
ED as % SVL	8.69	8.47-8.97	.257	5	7.49	7.03-8.25	.468	5	.036
Post Rostrals	10.4	10-11	.548	5	11.2	10-13	1.09	9	.216
Post Mentals	14.3	12-16	2.06	4	14.6	12-20	2.30	9	.770
Transtubercular W	6.78	2.60-10.4	3.06	6	5.80	3.80-7.80	1.28	9	.203
TtW as % SVL	11.5	5.67-15.5	3.40	6	8.56	7.17-11.8	1.49	9	.0089
Transiliac W	5.62	4.50-6.45	1.01	5	8.64	6.26-10.7	1.70	5	.017
TiW as % SVL	11.0	9.95-11.6	.940	5	11.6	10.4-13.4	1.13	5	.178
Spur Tubercles	20.8	12-26	5.54	5	25.7	12-40	10.4	6	.188
Body Width	10.5	7.0-12.6	3.06	4	16.8	12.4-21.9	3.08	6	.012
BW as % SVL	20.3	18.0-23.3	2.78	4	21.9	18.7-24.6	2.22	6	.181
Fat Body*	1.33	1-2	.58	3	3.33	2-4	1.41	3	.074
Lt Testis Length	3.71	1.80-5.50	1.40	5					
L T Width	1.94	.880-3.20	.830	5					
L T Depth	1.28	.040-2.70	.983	5					
L T Volume	7.78	.033-24.9	9.91	5					
Rt Testis Length	3.89	1.66-5.70	1.50	5					
R T Width	2.40	.920-4.00	1.17	5					
R T Depth	1.12	.060-1.80	.661	5					
R T Volume	8.02	.048-21.5	8.13	5					
TVol. Lt+Rt	15.8	.081-46.4	18.0	5					
Lt Follicle 1					4.02	2.40-7.00	2.58	3	
L F 2					2.30	2.30		2	
L F 3					1.85	1.85		1	
Rt Follicle 1					3.23	2.98-3.7	0.41	3	
R F 2					2.36	1.41-3.23	0.92	3	
R F 3					1.9	1.9		1	
L+R F as %SVL					?				

Table A1.3 (Continued).

N. DELEANI									
CHARACTER	MALES				FEMALES				*T*-TEST
	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	
Rostral Width	2.00	1.77-2.23	.237	4	2.38	1.93-2.94	.390	5	.339
RW as % SVL	3.46	3.44-3.47	.197	4	3.07	2.30-3.54	.504	5	.142
R Height	0.80	0.74-0.86	,083	4	.882	0.74-1.00	.103	5	.177
RH as % SVL	1.37	1.33-1.45	.069	4	1.127	.892-1.39	.204	5	.049
Mental Width	2.28	2.16-2.40	.139	4	2.49	1.78-2.87	.442	5	.280
MW as % SVL	3.97	3.70-4.24	.355	4	3.36	2.65-3.97	.472	5	.085
M Height	.795	0.63-0.96	.203	4	0.85	0.70-1.04	.133	5	.348
MH% as % SVL	1.40	1.24-1.48	.143	4	1.18	.854-1.51	.316	5	.153
R+M Area	3.45	2.67-4.22	.916	4	4.05	3.08-4.97	.699	5	.201
/(RMA)	1.88	1.63-2.07	.216	4	2.01	1.75-2.23	.176	5	.184
/(RMA) as % SVL	3.19	3.17-3.20	.068	4	2.73	2.38-3.06	.287	5	.027
Internasals	14	14	0	4	15.3	14-17	1.11	7	.090
Interorbitals	5	5	0	5	5.00	4-6	.707	9	1.00
Nasolabials	7	6-8	.632	8	7.00	5-8	.877	14	.576
Perinasals	18.6	17-20	1.13	7	22	21-24	1.27	6	.0013
Nasooculars	23.3	23-24	.577	5	24	21-26	2.12	5	.624
Supralabials	18.5	18-19	0.58	6	19.1	17-22	1.66	10	.504
Infralabials	19.0	18-21	1.22	7	20.6	17-24	1.96	10	.546
Transpalpebral	7.75	7-8	.500	4	9.0	8-10	1.0	5	.084
Palpebral Edge	33.0	29-36	2.94	4	34.0	28-39	4.47	5	.678
Midbody Scales	242	224-254	12.5	5	248	227-260	14.7	4	.574
Rostral-Pelvis Sc	358	339-376	16.2	5	365	344-376	14.6	4	.450
Mental-Vent Sc	379	362-407	18.0	5	376	362-385	12.5	3	.684
Rosette Scales	10.7	9-11	.577	4	10	10	0	5	NA
Inter-R Scales		0-4		4		0-3		5	NA
Knob Width	1.98	1.0-2.40	0.66	4	2.32	1.33-2.72	0.42	10	.126
Knob Height	1.68	0.80-2.20	0.61	4	1.84	1.00-2.50	0.50	10	.307
Knob Length	2.43	1.50-2.80	0.62	4	2.97	2.08-3.66	0.41	10	.033
Knob Volume	5.04	0.63-7.74	3.07	4	7.12	1.58-12.1	3.43	10	.156
3/KV	1.61	.856-1.98	.511	4	1.87	1.16-2.30	.349	10	.146
3/KV as %SVL	2.74	2.20-2.960	.365	4	2.74	2.03-3.93	.627	10	.492
Isthmus Width	0.85	0.50-1.20	.436	3	1.29	0.83-1.90	.031	8	.071
I W as % SVL	1.69	1.28-1.93	0.35	3	1.91	1.35-2.53	.448	8	.176
Caudal Annuli	15.7	15-16	.580	3	16	15-17	.707	8	.646
Knob Scales	41.9	39-46	2.70	8	40.5	37-44	4.95	2	.095
Forelimb Length	22.4	15.0-26.2	4.61	5	27,2	18.0-31.4	4.61	9	.034
FL as % SVL	39.0	36.1-40.8	1.89	5	37.9	33.2-43.6	3.44	9	.294
Hindlimb Length	27.8	18.5-32.4	6.26	5	33.7	22.6-40.0	6.40	9	.035
HL as % SVL	48.2	47.1-49.5	1.03	5	47.0	40.5-51.2	3.70	9	.259
Subdigital Lamellae	NA								
Latitudinal Rangee (m+f)		31.4-31.5°S							
Longitudinal Range (m+f)		137.1-137.3°E							

Table A1.4 Morphometric Data Analysis For *N. laevissimus*

CHARACTER	MALES				FEMALES				
	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Collection Dates		8.5-6.5				7.1-6.3			
SVL	60.0	33.6-80.6	11.3	131	68.3	35.2-94.7	13.7	129	<.0001
Tail Length	19.7	10.0-27.8	3.9	108	21.8	12.0-31.0	4.45	104	.00010
Total Length	79.3	37.6-107	15.8	108	89.6	49-124	18.3	104	NA
Tail as % SVL	33.0	24.5-42.9	3.24	108	32.4	19.3-46.3	3.44	104	.034
Head Width	15.2	8.8-21.8	2.87	131	17.1	9.00-23.1	3.18	128	<.0001
HW as % SVL	25.3	23.3-31.1	1.14	131	25.3	11.1-44.1	2.61	128	.372
Head Height	8.90	5.3-11.7	1.52	99	9.88	5.4-13.4	1.84	102	<.0001
HH as % SVL	14.6	8.52-17.1	0.99	99	14.7	13.0-18.2	0.86	102	.082
Head Length	17.2	14.6-21.1	2.24	6	19.6	12.6-22.5	3.17	10	.062
HL as % SVL	28.3	23.5-30.1	2.50	6	30.4	26.4-35.8	2.75	10	.083
Tail Width	6.04	2.90-8.40	1.40	78	6.48	2.8-9.9	1.72	80	.040
TW as % SVL	9.84	7.10-13.2	1.34	78	9.41	6.33-12.9	1.46	80	.021
Tail Height	4.11	2.00-5.70	0.90	78	4.42	6.10-2.10	1.02	79	.022
TH as % SVL	6.73	5.20-9.12	0.80	76	6.46	4.32-8.02	.802	79	.018
Tail Volume	144	21.3-330	73.2	76	186	23-443	100	79	.0018
3/TV	5.05	2.77-6.91	1.03	76	5.47	2.84-7.62	1.18	79	.0093
3/TV % SVL	8.28	6.79-10.4	.794	76	7.99	9.80-6.58	.727	79	.0093
Peduncle	6.92	3.7-12.5	1.57	81	7.52	4.20-12.1	1.81	75	.040
Ped. As % SVL	11.57	7.69-16.9	2.08	81	11.0	8.24-16.0	1.68	75	..040
Ear Height					2.31	1.55-3.3	0.83	4	
EH as % SVL					4.31	3.66-4.83	0.49	4	
Eye Diameter	5.03	3.3-6.80	0.81	95	5.45	3.30-7.40	0.94	94	.00071
ED as % SVL	8.38	7.23-10.1	0.59	95	8.05	6.71-10.1	0.63	94	.00014
Post Rostrals	13.3	9-20	2.19	99	12.9	8-19	2.15	98	.192
Post Mentals	15	11-19	1.96	99	15.3	11-22	2.28	98	.192
Transtubercular W	8.13	3.00-12.8	2.45	127	7.12	2.9-10.3	1.84	122	.00038
T tW as % SVL	13.2	7.14-17.3	2.28	127	10.3	7.18-16.9	1.29	122	<.0001
Transiliac W	7.45	3.6-10.1	1.47	98	8.55	3.8-12.1	1.93	96	<.0001
TiW as % SVL	12.2	10.4-14.1	0.75	98	12.6	10.2-14.3	0.75	96	.00023
Spur Tubercles	22.8	15-32	6.41	8	9.6	4-17	5.94	5	.0018
Body Width	15.8	13-22.2	3.38	8	14.3	9.16-19.9	4.74	6	.254
BW as % SVL	25	19.7-31.7	3.54	8	24.2	21.6-26.4	2.08	6	.315
Fat Body	3.11	1-5	0.65	88	3.05	1-5	0.83	84	.432
Lt Testis Length	4.37	0.5-7.2	1.65	124					
L T Width	2.85	0.3-5.40	1.14	123					
L T Depth	1.97	0.15-3.6	0.86	123					
L T Volume	17.8	0.02-62.3	12.5	123					
Rt Testis Length	4.29	0.50-6.90	1.60	123					
R T Width	3.01	0.29-1.24	1.24	123					
R T Depth	1.96	0.15-3.90	0.89	123					
R T Volume	18.6	0.02-70.8	14.0	123					
L+R TVol	36.3	0.03-111	25.2	123					
Lt Follicle 1					4.83	0.30-22.0	5.26	90	
L F 2					1.07	0.20-4.20	0.91	79	
L F 3					1.57	0.2-3.50	0.66	66	
Rt Follicle 1					4.7	0.27-23.8	5.14	89	
R F 2					2.0	0.2-4.70	0.99	84	
R F 3					1.52	0.20-2.70	0.62	71	
L+R F as %SVL					12.5	1.60-54.2	12.3	90	

Table A1.4 (Continued)

N. LAEVISSIMUS									
CHARACTER	MALES				FEMALES				
	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Rostral Width	2.09	1.84-2.34	.173	6	2.31	1.69-2.91	.492	9	.158
RW as % SVL	3.47	2.83	4.04	6	3.78	3.15-4.80	.570	9	.145
R Height	.750	.630-.860	.081	6	.898	.520-1.31	0.264	9	.105
RH as % SVL	1.25	.913-1.56	.207	6	1.45	.961-1.86	.272	9	.073
Mental Width	2.07	1.78-2.67	.342	6	2.21	1.63-2.86	0.427	9	.256
MW as % SVL	3.40	2.92-3.81	.299	6	3.63	3.08-4.69	0.494	9	.170
M Height	.807	.660-1.01	.129	6	0.868	.580-1.17	0.220	9	.276
MH as % SVL	1.33	1.12-1.51	.167	6	1.41	1.18-1.89	.231	9	.242
R + M Area	3.25	2.70-3.89	.498	6	4.17	1.85-6.42	1.79	9	.124
/(RMA)	1.80	1.64-1.97	.138	6	2.00	1.36-2.53	.452	9	.161
/(RMA) as % SVL	2.98	2.73-3.26	.229	6	3.25	2.61-3.89	.443	9	.092
Internasals	15.6	13-19	1.41	48	15.2	11-20	1.66	64	.130
Interorbitals	5.65	4-8	0.77	66	5.28	4-7	0.81	76	.0057
Nasolabials	6.31	5-8	0.79	62	6.4	4-9	0.81	103	.913
Perinasals	18.6	17-21	1.67	13	18.4	16-21	1.79	19	.227
Nasooculars					24.5	24-25	NA	2	NA
Supralabials	20.4	16-29	3.23	11	20.6	17-25	2.10	25	.761
Infralabials	20	19-22	1.00	11	21	17-25	2.22	27	.162
Transpalpebral	8.8	8-9	0.45	5	8.67	8-9	0.50	9	.630
Palpebral Edge	32.6	29-35	2.3	5	33.9	32-36	1.45	9	.219
Midbody Scales	267	240-291	19.7	5	265	242-283	14.3	6	.840
Rostral-Pelvis Sc	372	341-396	23.6	5	355	320-387	24.7	6	.287
Mental-Vent Sc	407	392-437	20.5	4	347	336-358	15.6	2	.023
Rosette Scales	8.8	8-9	NA	7	9.00	8-10	NA	5	NA
Inter-R Scales	NA	0-5	NA	7	NA	0-5	NA	5	NA
Knob Width	2.15	1.60-3.00	0.34	102	2.44	1.6-3.5	0.45	96	<.0001
Knob Height	1.85	1.1-2.8	0.33	102	2.1	1.00-2.90	0.370	96	<.0001
Knob Length	2.47	1.5-3.6	0.40	102	2.68	1.7-3.80	0.48	96	.0016
Knob Volume	43.4	15.7-118	19.2	102	61.0	15.7-135	27.0	96	<.0001
3/KV	3.44	2.50-4.91	.495	102	3.84	2.17-5.12	.634	94	<.0001
3/KV as % SVL	5.87	3.96-9.39	.932	102	5.67	2.69-8.74	.875	95	.074
Isthmus Width	1.35	0.90-1.90	.250	74	1.42	0.80-2.00	.260	79	.078
I W as % SVL	1.94	1.25-3.13	.548	74	2.41	2.25-2.57	..227	79	.00073
Caudal Annuli					16	15-17	0.710	5	NA
Knob Scales	38.5	36-41	2.38	4	43.8	39-55	7.63	4	.126
Forelimb Length	22.4	18.5-26.2	2.74	7	24.5	16.1-30.4	5.18	8	.184
FL as % SVL	36.8	34.9-38.1	1.28	7	38.6	35.1-45.7	3.37	8	.115
Hindlimb Length	28.7	23.8-35.3	4.41	7	30.2	18.8-36.8	6.3	8	.313
HL as % SVL	47.1	45.2-50.4	2.10	7	47.5	42.7-53.4	3.73	8	.225
Subdigital Lamellae	NA								
Latitudinal Range (m+f)		19.3-31.5°S							
Longitudinal Range (m+f)		121-135°E							

Table A1.5 Morphometric Data Analysis For *N. levis*

	MALES				FEMALES				
CHARACTER	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Collection Dates		7.5-6				9.5-6			
SVL	62.3	34-81	13.4	94	71.9	36.5-98	18.0	80	<.0001
Tail Length	26.1	13.8-37.0	6.62	78	29.3	14.3-43.3	7.90	49	.0025
Total Length	84.2	50-115	19.5	94	90.9	36.5-141	27.5	73	NA
Tail as % SVL	43.1	33.3-52.9	3.51	78	40.1	22-50	5.23	49	.0011
Head Width	16.7	9.61-21.0	2.81	87	18.5	9.0-23.9	4.27	80	.00042
HW as % SVL	26.1	18.6-34.3	2.38	87	25.9	21.5-31.4	1.63	80	.241
Head Height	9.71	5.5-12.1	2.13	14	10.3	7.18-13.4	2.08	14	.139
HH as % SVL	15.1	12.6-16.9	1.31	14	13.8	11.6-16.0	1.35	14	.023
Head Length	18.6	10.7-21.6	3.77	14	21.4	15.4-26.1	3.92	14	.021
HL as % SVL	28.8	27.4-31.5	1.51	14	28.8	26.2-31.2	1.69	14	.330
Tail Width	12.9	3.9-18.4	4.96	12	10.3	5.44-20.7	4.77	14	.213
TW as % SVL	19.4	11.5-25.4	4.37	12	13.6	7.99-25.4	5.21	14	.0096
Tail Height	6.47	2.13-8.8	2.27	12	5.59	3.11-9.30	1.95	14	.297
TH as % SVL	9.89	6.27-12.2	1.85	12	7.40	4.42-11.4	1.83	14	.0043
Tail Volume	757	35-1381	503	12	549	85.0-1391	453	14	.395
3/TV	8.42	3.26-11.1	2.82	12	7.61	4.40-11.2	2.28	14	.377
3/TV as % SVL	12.9	9.59-15.3	1.89	12	10.1	7.48-13.8	1.91	14	.0025
Peduncle	5.94	2.30-9.40	2.27	7	7.11	3.42-11.2	3.20	4	.248
Ped. as % SVL	89.19	5.54-12.6	2.38	7	9.18	6.92-13.8	3.21	4	.498
Ear Height	3.02	1.60-3.70	.759	13	3.27	2.13-4.53	.675	13	.107
EH as % SVL	4.66	3.45-5.39	.569	13	4.40	3.51-5.57	.588	13	.192
Eye Diameter	5.18	2.72-6.59	1.22	13	5.76	4.02-6.80	.924	13	.068
ED as % SVL	7.98	6.97-9.08	.616	13	7.81	6.30-9.68	1.02	13	.200
Post Rostrals	12.7	8-22	2.91	37	10.7	7-13	1.79	15	.020
Post Mentals	16	6-24	3.60	35	15	13-18	1.50	16	.306
Transtubercular W	9.38	3.15-13.1	2.67	45	7.04	3.5-12.6	2.62	31	.00031
T tW as % SVL	14.5	8.65-19.3	2.48	45	10.36	7.53-14.3	1.63	31	<.0001
Transiliac W	7.68	3.83-9.10	1.71	14	8.89	5.56-11.1	1.90	13	.017
TiW as % SVL	11.9	11.3-13.3	.581	14	11.84	11.0-13.6	0.90	13	.215
Spur Tubercles	17.8	15-21	2.17	7	14.6	8-22	4.33	10	.384
Body Width	17.3	8.77-21.7	4.42	11	17.4	10.7-24.1	4.67	11	.167
BW as % SVL	26.5	21.7-28.8	2.02	11	23.1	19.2-28.7	3.59	11	.152
Fat Body	4.5	3-6	.926	8	3.33	1-5	1.58	9	.140
Lt Testis Length	4.92	0.6-7.3	1.35	76					
L T Width	3.27	5.0-0.83	0.94	75					
L T Depth	2.29	0.4-3.9	0.81	75					
L T Volume	23.2	.302-54.2	13.9	75					
Rt Testis Length	4.88	0.71-7.5	1.37	73					
R T Width	3.49	0.7-5.9	1.14	73					
R T Depth	2.26	0.4-4.0	0.81	73					
R T Volume	24.7	0.26-74.9	16.8	73					
Lt+Rt TVol.	47.7	0.57-126	29.7	73					
Lt Follicle 1					5.86	0.12-26.2	6.18	54	
L F 2					2.94	1.00-8.57	2.57	7	
L F 3					2.02	1.45-4.67	1.41	5	
Lt F Volume					665	.0009-9417	1864	54	
Rt Follicle 1					6.78	0.11-27.7	7.66	52	
R F 2					2.65	0.85-5.32	1.65	7	
R F 3					1.82	0.50-3.39	1.02	6	
Rt. F Volume					1129	.0007-1129	2805	52	
3/ FV					6.69	0.13-23.6	7.07	54	
3/ FV as % SVL					8.04	0.27-27.4	7.82	54	

Table A1.5 (Continued)

N. LEVIS									
	MALES				FEMALES				
CHARACTER	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Rostral Width	2.76	2.36-3.07	.217	11	2.94	2.04-3.87	.571	10	.044
RH as % SVL	1.17	.958-1.49	.148	11	1.18	1.01-1.53	.162	11	.398
RW as % SVL	3.94	3.43-4.62	.358	11	3.82	2.49-4.68	.783	11	.179
R Height	.813	0.66-0.97	.102	11	.966	0.62-1.28	.184	11	.017
RH as % SVL	1.32	.958-1.57	.227	11	1.59	1.05-3.86	.865	11	.190
Mental Width	2.85	2.17-3.64	.441	11	2.90	1.69-3.77	.672	11	.320
MW as % SVL	4.02	2.55-6.62	1.23	11	3.80	2.76-5.02	.769	11	.183
M Height	0.924	0.66-1.12	.152	9	1.33	0.67-3.46	.884	9	.086
R + M Area	3.16	2.59-3.57	.290	9	3.26	2.67-4.13	.462	9	.335
/(RMA)	1.78	1.61-1.89	0.083	9	1.79	1.63-2.03	.120	9	.367
/(RMA) as % SVL	2.59	2.34-2.84	.207	9	2.28	1.76-3.24	.551	9	.080
Internasals	17.2	15-19	1.25	11	14.9	12-18	1.81	11	.022
Interorbitals	5	3-6	1.0	11	4.36	3-6	.924	13	.174
Nasolabials	6.45	5-8	.811	17	6.15	5-8	.938	27	.342
Perinasals	22.5	19-25	2.52	8	21.3	20-23	1.11	12	.592
Nasooculars	27.2	25-30	1.69	10	27.0	23-30	2.31	10	,937
Supralabials	18.1	14-20	1.45	19	18.2	14-21	1.33	9	.929
Infralabials	19.2	16-23	1.99	20	18.4	16-21	1.31	18	.053
Transpalpebral	7.46	6-10	1.04	11	7.57	6-9	.805	11	.770
Palpebral Edge	33.3	27-40	3.26	11	34.0	29-42	3.63	11	.627
Midbody Scales	259	248-269	7.74	6	259	249-274	9.79	8	.958
Rostral-Pelvis Sc	341	312-378	27.2	5	344	307-371	21.9	9	.707
Mental-Vent Sc	374	353-391	14.3	5	381	340-418	28.2	10	.604
Rosette Scales	9.36	9-10	.505	11	9.18	9-10	.303	11	NA
Inter-R Scales	NA	0-5		8	NA	0-5		9	NA
Knob Width	2.14	1.51-3.30	0.30	69	2.37	1.12-3.40	0.49	47	.00074
Knob Height	1.71	0.60-2.4	0.33	69	1.91	0.70-2.7	0.44	47	.0028
Knob Length	2.54	1.8-3.3	0.31	69	2.75	1.8-3.8	0.49	47	.0018
Knob Volume	5.04	1.8-10.6	1.94	69	7.12	1.36-16.9	3.85	47	<.0001
3/KV	1.69	1.22-2.20	.217	70	1.87	1.11-2.57	.356	48	.00042
3/KV as %SVL	2.73	1.79-4.83	.519	70	2.70	1.77-4.75	.615	48	.223
Isthmus Width	1.27	0.67-1.69	0.25	10	1.32	0.83-1.75	0.30	10	.287
I W as % SVL	2.01	1.72-2.99	0.39	10	1.73	1.17-2.51	0.36	10	.047
Caudal Annuli	17.6	16-19	0.90	10	18.0	17-19	0.62	10	.344
Knob Scales	38.2	32-42	3.46	9	39.6	34-46	3.40	9	.382
Forelimb Length	24.0	14.4-29.4	4.93	11	28.5	19.5-34.3	5.17	11	.016
FL as % SVL	37.3	33.2-42.6	3.29	11	38.6	33.1-56.8	6.76	11	.494
Hindlimb Length	29.5	18.2-37.7	6.41	11	34.7	25.4-41.4	6.07	11	.020
HL as % SVL	46.0	37.6-56.4	5.41	11	46.7	42.0-52.9	3.49	11	.401
Subdigital Lamellae	NA								
Latitudinal Range (m+f)		20.2-32.8°S		99					
Longitudinal Range (m+f)		129.4-145.9°E		99					

Table A1.6 Morphometric Data Analysis For *N. sheai*

CHARACTER	MALES				FEMALES				
	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Collection Dates		8.4-6.9		9		7.8-6.4		21	
SVL	74.1	47.4-100	15.1	11	96.9	58.9-121	18.5	21	.0011
Tail Length	13.7	10.8-20.1	3.41	10	15.3	7.8-21.1	3.82	20	.239
Total Length	88.1	58.6-120	17.0	11	111	69.1-141	21.4	21	.0019
Tail as % SVL	19.6	14.7-25.4	4.02	10	15.9	8.57-20.2	2.55	20	.0026
Head Width	20.9	13-27.9	4.25	11	25.9	14.1-33.8	5.22	21	.0052
HW as % SVL	27.8	26.2-30.0	1.07	11	26.7	23.9-29.0	1.46	21	.015
Head Height	11.8	8.0-15.9	2.10	11	14.4	8.4-18.3	2.69	21	.0041
HH as % SVL	15.8	14.7-18	1.00	11	14.9	13.3-17.2	1.00	21	.014
Head Length	21.1	15.2-27.1	3.26	10	27.2	18.0-33.3	4.33	20	.00026
HL as % SVL	29.2	27.7-32.1	1.38	10	27.9	25.6-30.9	1.43	20	.0098
Tail Width	5.28	3.2-8.9	1.64	10	5.1	3.0-6.52	1.00	20	.355
TW as % SVL	7.07	5.6-8.9	1.25	10	5.35	4.03-7.78	0.85	20	<.0001
Tail Height	3.79	2.29-6.40	1.10	10	4.02	2.3-5.7	0.87	20	.262
TH as % SVL	5.09	4.34-6.84	0.86	10	4.18	3.53-5.14	0.47	20	.00038
Tail Volume	88.2	21.5-298	79.9	10	88.5	19-149	42.2	20	.494
3/ TV	4.08	2.78-6.68	1.05	10	4.32	2.67-5.31	0.82	20	.250
3/TV as % SVL	5.53	4.56-7.17	.840	10	4.51	3.62-5.51	.410	20	<.0001
Peduncle	3.06	1.90-4.00	0.55	10	3.83	2.1-8.1	1.30	20	.043
Ped. As % SVL	4.28	2.57-6.69	1.17	10	4.19	2.43-13.0	2.25	20	.454
Ear Height	3.86	2.24-5.30	0.74	10	4.99	2.8-6.6	1.17	18	.0053
EH as % SVL	5.33	4.73-6.24	0.52	10	5.04	4.35-6.01	0.55	18	.091
Eye Width	5.28	4.09-6.90	0.81	10	6.50	4.40-8.00	1.02	18	.0017
EW as % SVL	7.34	6.64-8.63	0.58	10	6.68	5.18-8.68	0.86	18	.020
Post Rostrals	12.6	9-17	2.91	11	11.4	7-15	1.78	21	.154
Post Mentals	10.5	7-14	2.16	11	9.86	7-13	1.42	21	.354
Transtubercular W	8.98	3.80-14.9	3.21	11	9.24	6.40-13.1	2.24	19	.399
TtW as % SVL	11.7	8.02-15.7	2.45	11	9.55	6.14-11.3	1.51	19	.003
Transiliac W	8.76	5.71-11.7	1.83	10	12.2	6.0-15.0	2.72	18	.00083
TiW as % SVL	12.1	10.5-13.6	0.95	10	12.2	10.2-13.7	1.03	18	.334
Spur Tubercles	14	14		1	14.4	8-21	4.04	6	.924
Body Width	17.0	9.66-23.5	4.06	10	24.0	13.1-38.7	8.13	20	.0085
BW as % SVL	23.6	17.3-31.2	4.71	10	24.2	12.6-33.3	5.87	20	.382
Fat Body	3.4	1-6	1.65	10	2.13	1-5	1.25	15	.019
Lt Testis Length	4.36	2.7-6.30	1.36	10					
L T Width	3.18	1.51-4.6	1.20	10					
L T Depth	2.19	0.93-3.5	0.92	10					
L T Volume	21.3	1.99-51.1	19.2	10					
Rt Testis Length	4.34	2.35-6.5	1.58	10					
R T Width	3.37	2.11-4.50	0.93	10					
R T Depth	2.14	0.76-3.50	0.91	10					
R T Volume	21.7	2.04-51.1	19	10					
L+R TVol	43.0	4.03-102	38	10					
Lt Follicle 1					9.58	0.53-28.1	10.1	14	
L F 2					2.65	0.84-3.90	1.00	6	
L F 3					1.50	0.23-2.05	0.85	4	
Rt Follicle 1					9.61	0.53-30.9	10.7	14	
R F 2					1.94	0.41-3.20	1.14	6	
R F 3					1.22	0.40-2.07	0.94	4	
Lt+Rt Fol					19.2	1.06-59.0	20.8	14	
L+R F as %SVL					17.8	1.80-53.0	18.4	14	

Table A1.6 (Continued).

N. SHEAI									
CHARACTER	MALES				FEMALES				
	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Rostral Width	3.52	3.00-4.60	0.54	8	4.00	2.65-5.2	0.81	12	.077
RW as % SVL	4.45	3.70-5.41	0.55	8	4.13	3.49-4.72	0.34	12	.066
R Height	1.03	0.82-1.30	0.18	8	1.21	0.77-1.58	0.25	12	.057
RH as % SVL	1.31	1.10-1.64	.205	8	1.24	1.12-1.41	.109	12	.187
Mental Width	2.42	1.90-3.17	0.38	8	2.69	1.87-3.29	0.40	12	.074
MW as % SVL	3.08	2.30-3.88	0.55	8	2.83	2.18-3.79	0.51	12	.156
M Height	0.82	0.61-1.10	0.17	8	0.91	0.56-1.25	0.18	12	.149
MH as % SVL	1.03	.827-1.24	.130	8	.948	.722-1.25	.161	12	.118
R + M Area	5.68	4.17-8.28	1.52	8	7.46	3.36-10.5	2.40	12	.040
/(RMA)	2.37	2.04-2.88	.306	8	2.69	1.83-3.24	.474	12	.051
/(RMA) as % SVL	2.99	2.69-3.59	.280	8	2.80	2.48-3.25	.247	12	.058
Internasals	19.5	15-22	2.34	11	17.8	14-22	1.94	21	.033
Interorbitals	10.1	6-13	1.81	11	9.95	8-13	1.31	19	.804
Nasolabials	5.05	4-8	1.16	22	4.67	4-7	0.85	42	.224
Perinasals	21.4	20-25	1.77	8	20.5	18-24	1.62	17	.250
Nasooculars	31.3	29-33	1.32	9	31.7	26-38	3.77	10	.810
Supralabials	16.0	13-19	1.63	20	16.4	14-21	1.81	26	.585
Infralabials	16.5	14-21	1.92	20	16.8	14-21	1.83	26	.747
Transpalpebral	7.18	6-8	0.75	11	7.53	6-11	1.12	19	.374
Palpebral Edge	29.8	25-32	1.93	10	29.3	23-34	2.67	18	.592
Midbody Scales	331	307-385	36.8	4	308	288-336	21.6	4	.322
Rostral-Pelvis Sc	396	255-465	95.3	4	446	427-459	13.5	4	.337
Mental-Vent Sc	346	308-387	35	4	358	341-388	20.9	4	.585
Rosette Scales	6.89	6-8	0.60	9	6.72	6-8	0.67	18	.534
Inter-R Scales		2-10		9		0-12		18	NA
Knob Width	3.69	3.01-4.70	0.52	9	4.06	2.85-5.74	0.64	18	.076
Knob Height	2.83	2.08-4.00	0.58	9	3.18	1.81-4.00	0.59	18	.073
Knob Length	3.05	2.48-4.00	0.43	10	3.36	2.56-4.26	0.52	18	.063
Knob Volume	17.7	8.89-39.4	9.34	9	24.0	6.91-49.4	10.5	18	.071
3/KV	2.51	2.07-3.40	.410	9	2.83	1.91-3.67	.430	18	.041
3/KV as % SVL	3.57	2.80-4.47	.567	9	2.94	258-3.59	.290	18	.00038
Isthmus Width	1.25	0.99-1.80	0.25	10	1.39	0.92-1.90	0.24	18	.076
I W as % SVL	1.72	1.14-2.09	.270	10	1.45	1.10-1.81	0.18	18	.0020
Caudal Annuli	9.67	8.5-11	0.83	9	9.53	8-11.5	0.96	18	.358
Knob Scales	41.1	39-45	2.03	8	44.6	35-51	4.18	17	.038
Forelimb Length	32.5	21.5-42.4	5.50	10	40.2	23.1-49.0	6.99	20	.0024
FL as % SVL	44.9	41.9-48.1	1.94	10	41.1	36.7-45.2	2.32	20	<.0001
Hindlimb Length	36.0	24.5-45.9	5.80	10	45.9	25.0-57.5	8.19	20	.00099
HL as % SVL	49.8	47.9-52.1	1.67	10	46.9	42.4-50.1	2.03	20	.00023
Subdigital Lamellae	NA								
Latitudinal Range (m+f)		12.5-18.7°S							
Longitudinal Range (m+f)		124.9-133.1°E							

Table A1.7 Morphometric Data Analysis For *N. stellatus*

	MALES				FEMALES				
CHARACTER	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TESTS
Collection Dates		9.8-4.4		11		10.1-5.0		10	
SVL	62.0	51.7-78.0	7.95	19	64.3	46.5-86.0	13.9	13	.277
Tail Length	21.0	17.1-27.3	2.65	18	20.1	15.1-27	3.48	12	.216
Total Length	82.3	61.0-103	10.6	19	82.9	62.4-113	17.0	13	.455
Tail as % SVL	33.8	26.6-39.0	3.94	18	32.0	27.1-35.8	2.89	12	.094
Head Width	16.4	13.0-19.5	1.95	19	17.1	11.9-21.3	3.16	12	.229
HW as % SVL	26.6	23.8-30.3	1.69	19	26.2	24.1-28.7	1.46	12	.246
Head Height	8.01	6.78-9.60	.825	16	7.72	5.50-9.50	1.27	9	.250
HH as % SVL	13.3	11.5-15.3	1.22	16	13.0	11.2-14.4	1.08	9	.230
Head Length	18.0	15.5-19.3	1.24	8	20.5	19.2-23.1	2.22	4	.018
HL as % SVL	30.3	28.0-33.5	1.87	8	31.0	29.5-31.7	1.24	4	.285
Tail Width	5.43	4.30-6.90	.856	15	4.92	3.50-6.30	1.00	9	,105
TW as % SVL	8.93	6.73-10.8	1.03	15	8.43	7.13-9.36	.819	9	.126
Tail Height	3.78	3.08-4.67	.569	15	3.51	2.60-4.51	.751	9	.173
TH as % SVL	6.21	5.15-7.60	.662	14	6.03	4.42-6.94	.831	9	.290
Tail Volume	120	62.5-218	48.3	14	91.6	39.3-140	43.7	9	.093
3/TV	4.85	3.97-6.01	.646	14	4.40	3.40-5.19	.749	9	.076
3/TV as % SVL	7.93	6.50-9.37	.652	14	7.57	6.65-8.40	.529	9	.103
Peduncle	6.45	5.25-7.90	1.19	4	6.85	6.70-6.90	.100	5	.264
Ped. as % SVL	10.5	8.22-12.7	2.39	4	8.58	8.02-9.65	.732	5	.084
Ear Height	2.48	2.19-2.98	.244	14	2.46	2.18-2.70	.185	9	.456
EH as % SVL	4.10	3.29-4.77	.388	14	3.51	3.01-4.43	.484	9	.0025
Eye Diameter	4.97	4.00-5.65	.553	16	4.58	3.90-6.00	.709	10	.064
ED as % SVL	8.26	7.40-9.67	.709	16	7.05	4.67-8.92	1.61	10	.0068
Post Rostrals	10.3	8-12	1.18	15	12.0	10-17	2.33	9	.032
Post Mentals	14.7	13-18	1.62	15	14.9	11-20	2.57	10	.857
Transtubercular W	7.70	4.75-11.2	1.87	19	6.75	4.00-9.50	1.69	13	.082
TtW as % SVL	12.3	8.84-14.4	1.88	19	10.2	8.60-11.8	.866	13	.00056
Transiliac W	7.92	7.21-9.10	.633	14	8.16	6.11-10.2	1.30	7	.292
TiW as % SVL	13.1	11.9-14.4	.698	14	13.1	11.4-14.0	.937	7	.478
Spur Tubercles	28	20-38	4.55	10	28.4	18-35	6.88	6	.894
Body Width	16.1	12.3-20.8	2.33	13	16.0	13.1-19.1	2.26	9	.486
BW as % SVL	26.8	21.4-31.0	2.64	13	27.0	24.7-29.3	1.46	9	.432
Fat Body	3.60	2-5	1.52	5				0	
Lt Testis Length	4.68	2.49-6.44	1.76	6					
L T Width	2.70	0.97-4.00	1.11	6					
L T Depth	1.85	0.64-2.90	1.09	6					
L T Volume	18.1	.809-38.3	16.9	6					
Rt Testis Length	4.69	2.65-6.51	1.79	6					
R T Width	2.84	1.40-4.10	1.16	6					
R T Depth	1.69	0.32-2.80	1.02	6					
R T Volume	17.8	.673-37.9	16.7	6					
Lt+Rt TVol	35.9	1.48-76.1	33.6	6					
Lt Follicle 1					8.35	8.00-8.70		2	
LF Volume					306	268-344		2	
Rt Follicle 1					9.00	8.80-9.20		2	
L+R F Volume					689	675-701		2	
LRFV as % SVL					20.5	20.0-21.1		2	

Table A1.7 (Continued).

N. STELLATUS									
CHARACTER	MALES				FEMALES				
	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Rostral Width	2.54	2.16-2.91	.273	5	2.67	2.57-2.77	.108	4	.272
RW as % SVL	4.26	3.87-5.36	.621	5	4.01	3.80-4.22	.369	4	.168
R Height	.896	0.67-1.15	.171	9	.893	0.72-1.11	.192	4	.326
RH as % SVL	1.48	1.06-2.01	.286	9	1.39	1.17-1.52	.159	4	.170
Mental Width	2.32	2.12-2.64	.197	5	2.40	2.15-2.65	.250	3	.316
MW as % SVL	3.88	3.51-4.18	.275	5	3.58	3.53-3.64	.217	3	.035
M Height	1.13	0.82-1.32	.166	10	1.10	0.82-1.31	.252	4	.242
MH as % SVL	1.86	1.36-2.28	.296	10	1.76	1.33-2.01	.304	4	.379
R + M Area	5.45	3.27-6.25	1.21	5	5.62	4.70-6.55	1.31	3	.204
/(RMA)	2.26	1.81-2.50	.282	5	2.42	2.17-2.56	.221	3	.193
/(RMA) as % SVL	3.77	3.37-4.51	.455	5	3.49	3.41-3.56	.078	3	.108
Internasals	17.2	14-19	1.42	16	16.7	14-19	1.73	9	.425
Interorbitals	5.31	4-7	.793	16	5.00	3-6	.866	9	.369
Nasolabials	5.58	3-8	1.16	32	6.00	5-7	.699	8	.159
Perinasals	20.1	16-24	2.87	13	19.8	18-21	1.30	6	.840
Nasooculars	22.1	20-24	1.27	13	22.3	20-24	1.71	5	.827
Supralabials	17.2	14-21	1.63	19	16.5	15-19	1.64	7	.169
Infralabials	18.0	14-21	1.73	20	17.1	15-19	1.53	7	.193
Trans Palpebral	8.93	8-10	.475	14	8.67	8-10	.816	7	.375
Palpebral Edge	31.1	27-33	2.03	10	32.5	30-36	2.65	5	.303
Midbody Scales	254	218-289	24.7	9	249	244-254		4	.773
Rostral-Pelvis Sc	347	315-389	32.9	6	368			2	.580
Mental-Vent Sc	347	321-373	19.7	5	308			2	.147
Rosette Scales		8-11		12		9-11		5	NA
Inter-R Scales		1-9		5		4-9		2	NA
Knob Width	3.00	2.20-4.90	.635	16	3.30	2.05-4.90	.876	11	.159
Knob Height	2.57	1.76-4.60	0.81	16	2.93	1.77-4.6	1.07	11	.165
Knob Length	2.98	2.31-3.33	.306	16	3.10	2.7-3.6	.246	11	.156
Knob Volume	12.9	4.76-39.0	8.64	16	17.1	5.89-39.0	11.2	11	.138
3/KV	2.26	1.68-3.39	.429	16	2.48	1.81-3.39	.524	11	.130
3/KV as % SVL	3.64	2.93-4.43	.449	16	3.83	3.08-4.66	.393	11	.137
Isthmus Width	1.26	1.04-1.42	.116	13	1.24	0.90-1.40	.161	8	.368
I W as % SVL	2.11	1.62-2.41	.238	13	2.14	1.82-2.51	.226	8	.358
Caudal Annuli	12.8	11-14	.832	13	12.8	11-15	1.33	7	.449
Knob Scales	36.7	32-39	4.04	3	41	41		1	.451
Forelimb Length	23.6	19.9-26.4	1.96	15	23.0	18.4-28.8	3.45	9	.210
FL as % SVL	39.5	34.8-43.2	2.47	15	38.6	36.3-41.5	1.84	9	.065
Hindlimb Length	28.7	25.2-34.7	2.77	15	27.4	21.5-33.8	4.33	9	.172
HL as % SVL	47.8	42.7-51.2	2.31	15	46.0	40.9-50.3	3.08	9	.022
Subdigital Lamellae	NA								
Latitudinal Range (m+f)		30.0-33.6°S		31					
Longitudinal Range (m+f)		119.0-137.1°E		31					

Table A1.8 Morphometric Data Analysis For *N. vertebralis*

	MALES				FEMALES				
CHARACTER	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Collection Dates		9.5-5.1		28		9.5-5.5		10	
SVL	60.39	39.9-77.7	10.61	34	69.1	40.9-90.0	17.9	14	.021
Tail Length	22.5	17.1-28.5	3.32	29	26.9	15.7-33.5	5.81	14	.0015
Total Length	80.7	57.2-101	13.0	30	92.4	45.8-121	25.8	13	.027
Tail as % SVL	38.8	33.5-46.1	3.01	29	38.1	33.1-44.9	2.99	12	.250
Head Width	14.5	9.10-19.5	2.57	33	16.5	9.20-21.2	4.03	14	.024
HW as % SVL	24.0	22.3-27.4	1.28	33	24.0	21.0-28.3	1.88	14	.464
Head Height	8.56	6.0-10.2	1.27	29	9.61	6.1-12.6	2.26	13	.031
HH as % SVL	14.2	12.0-16.3	1.03	29	14.4	12.8-17.0	1.11	12	.371
Head Length	17.2	12.0-20.7	2.56	28	18.8	11.1-23.0	4.41	13	.076
HL as % SVL	28.4	25.9-30.8	1.19	28	27.9	24.7-30.9	1.81	13	.137
Tail Width	6.60	3.30-13.2	2.17	28	9.15	3.70-16.8	3.74	13	.0043
TW as % SVL	11.2	8.23-18.3	2.31	28	12.7	9.05-21.6	3.77	13	.062
Tail Height	4.31	2.50-6.70	1.04	28	5.59	2.70-8.80	1.82	13	.0033
TH as % SVL	7.40	5.67-9.28	.831	28	7.81	6.03-11.6	1.54	13	.138
Tail Volume	191	36.9-630	137	28	447	41-1297	361	13	.0010
3/TV	5.49	3.33-8.57	1.25	28	7.08	3.45-10.9	2.16	13	.0024
3/TV as % SVL	9.37	7.81-11.9	.984	28	9.91	8.44-14.3	1.73	13	.105
Peduncle	9.00	6.40-13.2	1.70	22	9.92	5.70-12.6	2.10	13	.084
Ped. as % SVL	15.3	11.9-19.4	2.00	22	14.5	11.5-17.4	2.08	12	.129
Ear Height	2.53	1.60-3.30	.495	28	3.00	1.70-4.10	.742	13	.010
EH as % SVL	4.16	3.42-4.89	.406	28	4.47	3.92-5.94	.588	13	.028
Eye Diameter	4.95	3.70-6.20	.745	28	5.16	3.40-7.10	1.20	11	.259
ED as % SVL	8.21	7.25-9.60	.611	28	7.86	6.44-8.96	.753	11	.069
Post Rostrals	9.48	6-13	1.90	29	8.62	7-10	1.26	13	.142
Post Mentals	12.2	6-18	2.56	29	11.7	8-15	1.97	13	.553
Transtubercular W	7.58	3.00-11.2	2.33	29	6.67	3.40-10.1	2.19	13	.276
TtW as % SVL	12.2	7.48-15.7	2.14	29	9.56	7.74-11.9	1.24	13	.00023
Transiliac W	6.34	3.9-8.3	1.12	29	7.31	3.7-9.9	2.27	12	.073
TiW as % SVL	10.5	9.12-12.3	.663	28	10.7	9.05-12.3	.832	12	.424
Spur Tubercles					15			1	
Body Width	11.6	6.5-18.6	2.56	28	14.2	8.5-19.1	4.15	12	.011
BW as % SVL	19.1	14.8-24.3	2.39	28	20.8	19.8-22.3	.666	12	.0098
Fat Body	3.44	0-5	1.34	32	3.75	1-5	1.22	12	
Lt Testis Length	4.52	1.66-6.60	1.42	33					
L T Width	2.73	0.72-4.55	1.02	33					
L T Depth	1.77	0.38-3.11	.769	33					
L T Volume	14.5	.318-34.8	11.3	33					
Rt Testis Length	4.53	1.36-6.78	1.38	33					
R T Width	2.84	0.91-4.50	1.00	33					
R T Depth	1.72	0.31-3.10	.669	33					
R T Volume	14.7	.261-34.7	10.6	33					
Lt+Rt Testes Vol.	29.2	0.58-68.8	21.8	33					
Lt Follicle 1					3.99	0.20-13.2	4.25	13	
LF 2					2.38	0.38-5.90	1.67	10	
LF 3					1.93	0.48-4.80	1.32	9	
LF 4					1.14	0.23-1.79	.591	8	
Rt Follicle 1					4.32	0.78-13.6	3.91	13	
RF 2					2.18	0.39-4.9	1.32	11	
RF 3					1.63	0.30-2.72	.778	10	
RF4					1.24	0.42-1.79	.476	8	
L+R F as %SVL					4.77	0.98-12.0	3.28	12	

Table A1.8 (Continued).

N. VERTEBRALIS									
	MALES				FEMALES				
CHARACTER	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Rostral Width	2.15	1.62-2.56	.254	16	2.24	1.83-2.75	.294	11	.222
RW as % SVL	3.23	2.25-4.04	.433	16	3.14	2.45-4.35	.572	10	.332
R Height	.755	0.54-1.01	.125	16	.886	0.65-1.02	.113	10	.012
RH%	1.13	.845-1.41	.160	16	1.17	.941-1.44	.174	10	.259
Mental Width	2.54	1.95-3.48	.422	16	2.76	2.21-3.49	.502	9	.122
MW as % SVL	3.78	3.12-5.01	.502	16	3.62	2.82-4.27	.503	9	.226
M Height	.733	0.56-0.90	.094	16	.894	0.68-1.19	0.18	9	.003
RH%	1.09	.889-1.34	.115	16	1.17	.900-1.39	.184	9	.100
R + M Area	3.53	2.37-5.46	.838	16	4.54	2.87-6.51	1.27	9	.012
/(R+M Area)	1.87	1.54-2.34	.219	16	2.11	1.69-2.55	.299	9	.013
/(RMA) as % SVL	2.78	2.36	3.37	16	2.78	2.26	3.30	9	.476
Internasals	14.5	11-18	1.64	29	13.5	11-15	1.31	12	.083
Interorbitals	4.93	3-7	.998	29	4.42	3-6	.996	12	.141
Nasolabials	6.15	5-8	.812	39	5.55	4-7	.686	20	.0062
Perinasals	20.3	16-22	2.22	7	21.6	19-24	1.82	5	.302
Nasooculars	27.3	24-33	2.29	28	26.8	24-30	1.85	12	.550
Supralabials	20.2	17-23	1.67	56	20.0	16-23	1.41	24	.480
Infralabials	19.4	16-24	1.48	55	19.7	17-23	1.37	23	.555
Transpalpebral	8.82	8-10	.786	27	8.92	8-10	0.9	12	.723
Palpebral Edge	32.2	27-36	2.70	27	31.9	28-38	2.94	12	.811
Midbody Scales	243	228-259	14.1	7	236	212-258	19.5	4	.467
Rostral-Pelvis Sc	371	331-422	30.2	7	345	286-392	50.4	4	.303
Mental-Vent Sc	404	386-422	14.6	6	412	392-428	15.2	4	.422
Rosette Scales	9.57	8-11	.616	27	9.67	9-10	.492	12	.649
Inter-R Scales	2.17	2-3	.491	23	2.11	1-3	.333	9	NA
Knob Width	2.07	1.40-2.58	.317	27	2.45	1.77-3.11	.477	13	.0027
Knob Height	1.61	1.24-3.00	.423	27	1.81	1.32-2.90	.416	13	.188
Knob Length	2.11	1.60-2.84	.288	27	2.58	1.75-3.22	.488	13	.00024
Knob Volume	3.79	2.07-7.83	1.54	27	6.19	2.14-10.0	2.52	13	.00031
3/ KV	1.53	1.27-1.99	.196	27	1.80	1.29-2.15	.269	13	.00046
3/KV as % SVL	2.68	1.78-3.41	.421	27	2.61	2.08-3.67	.426	13	.302
Isthmus Width	1.04	0.74-1.41	.199	23	1.22	0.76-1.70	.315	12	.026
I W as % SVL	1.82	1.36-2.64	.308	23	1.73	1.33-2.08	.265	12	.197
Caudal Annuli	18.7	17-20	.863	23	18.5	17-20	.907	11	.459
Knob Scales	33.8	29-40	3.19	15	35.2	30-40	2.78	10	.270
Forelimb Length	23.8	17.4-28.8	3.64	27	25.3	15.6-32.0	5.49	13	.140
FL as % SVL	39.1	35.8-43.6	2.12	27	37.8	33.7-42.6	2.71	13	.040
Hindlimb Length	30.1	20.0-37.3	5.02	27	32.0	19.6-39.9	7.37	13	.141
HL as % SVL	49.2	44.5-54.1	2.56	27	47.6	43.2-52.3	2.99	13	.043
Subdigital Lamellae	NA								
Latitudinal Range (m+f)		25.0-30.6°S							
Longitudinal Range (m+f)		116.0-126.6°E							

Table A1.9 Morphometric Data Analysis For *N. wheeleri*

	MALES				FEMALES				
CHARACTER	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Collection Dates		7.5-6.5		8		7.1-6.5		12	
SVL	70.5	38.2-82.6	13.7	14	81.4	60.0-102.5	12.5	14	.076
Tail Length	26.7	16.4-31.4	6.92	4	31.0	23.2-42.5	5.71	9	.073
Total Length	79.6	39.9-112	17.9	14	102.6	71.3-145	19.7	14	NA
Tail as % SVL	40.4	39.0-41.7	1.21	4	39.1	34.1-42.5	2.68	9	.442
Head Width	18.3	10.3-21.6	3.44	14	20.5	16.3-23.8	2.19	14	.180
HW as % SVL	26.1	24.6-27.5	.860	14	25.4	23.1-27.8	1.49	14	.027
Head Height	9.75	5.70-11.4	1.82	13	11.0	8.7-12.7	1.27	14	.214
HH as % SVL	14.0	13.4-14.9	.493	13	13.5	12.2-15.8	.951	14	.194
Head Length	19.2	11.5-22.8	3.37	14	21.6	16.6-26.2	2.32	14	.177
HL as % SVL	27.4	25.7-30.1	1.27	14	26.7	24.2-29.8	1.74	14	.314
Tail Width	10.6	5.60-16.3	4.40	4	11.9	7.6-18.0	3.57	9	.322
TW as % SVL	15.72	13.7-20.2	3.06	4	14.8	9.81-17.7	2.51	9	.222
Tail Height	5.5	3.0-7.3	1.93	4	6.48	4.5-8.7	1.51	9	.399
TH as % SVL	9.20	7.12-9.32	1.44	4	8.14	5.81-9.67	1.12	9	.330
Tail Volume	497	72.1-978	380	4	714	226-1682	493	9	.262
3/TV	7.39	4.16-9.93	2.43	4	8.53	6.06-11.9	1.98	9	.261
3/TV as % SVL	11.1	10.2-12.3	1.05	4	10.7	8.19-12.0	1.19	9	.116
Peduncle	7.10	5.0-8.6	1.59	4	10.4	8.1-13.7	2.09	9	.0087
Ped. as % SVL	10.9	9.69-12.2	1.32	4	13.1	10.8-15.9	1.68	9	.021
Ear Height	3.18	1.6-4.0	.735	14	3.42	2.3-4.3	.619	14	.399
EH as % SVL	4.50	3.87-5.26	.452	14	4.21	3.31-5.16	.497	14	.098
Eye Diameter	5.06	3.2-6.1	0.90	11	5.74	4.6-7.7	.693	14	.173
ED as % SVL	7.42	6.58-8.78	.642	11	7.12	6.02-8.11	.709	14	.673
Post Rostrals	7.07	5-10	1.54	14	5.64	3-8	1.78	14	.076
Post Mentals	7.29	6-8	.726	14	7.5	6-9	1.02	14	.641
Transtubercular W	9.33	3.3-13.3	2.87	14	15.4	6.4-98.9	24.1	14	.022
TtW as % SVL	12.9	8.64-16.1	2.29	14	10.1	9.30-12.7	1.09	14	<.0001
Transiliac W	8.28	4.1-10.3	1.81	14	9.03	6.9-13.5	1.62	14	.144
TiW as % SVL	11.3	10.0-12.5	.742	14	11.5	10.3-13.2	.677	14	.250
Spur Tubercles				2				2	
Body Width	15.1	7.9-19.3	3.33	14	18.2	13.1-24.6	4.12	14	.113
BW as % SVL	21.4	18.2-24.7	1.85	14	22.3	18.5-26.9	2.66	14	.151
Fat Body	4.07	1-5	1.27	14	4.29	1-6	1.38	14	.277
Left Testis L	5.77	1.04-7.58	1.82	13					
L T Width	3.11	0.66-4.83	1.14	13					
L T Depth	1.81	0.43-2.94	.713	13					
L T Volume	20.6	.155-50.5	15.5	13					
Rt Testis Length	6.02	1.05-7.92	1.92	13					
R T Width	3.44	0.47-5.26	1.27	13					
R T Depth	1.96	0.35-3.02	.793	13					
R T Volume	25.3	0.09-52.6	15.6	13					
Lt+Rt TVol	45.9	.245-101	28.5	13					
Lt Follicle 1					5.30	1.29-18.2	5.43	14	
L F 2					2.49	0.93-3.82	1.01	14	
L F 3					1.74	0.57-2.64	.728	14	
Rt Follicle 1					5.29	1.24-18.8	5.72	14	
R F 2					2.35	1.0-3.7	.921	14	
R F 3					1.80	0.41-2.68	.793	14	
L+R F as %SVL					10.6	2.53-37.0	11.1	14	

Table A1.9 (Continued).

N. WHEELERI									
	MALES				FEMALES				
CHARACTER	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Rostral Width	2.11	1.61-3.10	.519	9	2.39	1.66-2.99	.559	6	.172
RW as % SVL	2.78	2.04-4.13	.726	9	2.77	1.74-3.46	.762	6	.498
R Height	.896	0.8-1.0	.081	9	.948	0.72-1.13	.157	6	.202
Mental Width	2.36	1.85-2.83	.327	9	2.43	1.85-2.93	.332	8	.334
MW as % SVL	3.08	2.35-3.59	.355	9	2.80	2.25-3.34	.328	8	.056
M Height	0.826	0.69-1.00	0.11	9	.978	0.77-1.17	0.12	8	.0078
R + M Area	3.88	3.13-5.04	.787	9	4.08	2.28-6.58	1.42	8	.359
/RMA	1.96	1.77-2.25	.196	9	1.99	1.51-2.57	0.35	8	.409
/RMA as % SVL	2.57	2.21-2.99	.275	9	2.30	1.67-2.62	.341	8	.043
Internasals	14.1	11-16	1.39	14	13.8	12-16	1.25	14	.585
Interorbitals	5.57	5-7	.756	14	5.50	4-7	1.02	14	.988
Nasolabials	3.92	3-5	.515	24	3.93	2-5	.829	28	.780
Perinasals	19.6	18-22	1.52	5	19.4	17-22	2.07	14	.866
Nasooculars	21.5	16-26	2.99	13	20.3	15-23	1.98	14	.085
Supralabials	18.9	15-22	1.59	23	19.9	16-22	1.08	28	.211
Infralabials	17.6	15-20	1.23	13	17.3	16-23	1.05	14	.054
Transpalpebral	7.62	6-9	.961	13	8.21	7-10	1.05	14	.126
Palpebral Edge	30.0	27-33	1.87	13	31.9	29-36	1.99	14	.052
Midbody Scales	285	267-304	17.2	6	288	264-310	17.1	8	.738
Rostral-Pelvis Sc	440	367-483	47.1	6	456	409-491	30.2	5	.544
Mental-Vent Sc	394	344-460	46.0	6	367	320-421	45.1	5	.344
Rosette Scales	9.14	8-10	.770	14	9.29	9-10	.469	14	.645
Inter-R Scales		0-12		7		1-17		4	NA
Knob Width	2.36	2.16-2.75	.267	4	2.41	1.93-3.09	.353	9	.267
Knob Height	1.47	1.31-1.76	.203	4	1.66	1.25-2.20	.312	9	.304
Knob Length	2.39	2.21-2.50	.125	4	2.98	2.33-4.24	.613	9	.050
Knob Volume	4.40	3.67-6.30	1.33	4	6.59	3.40-15.1	3.53	9	.139
3/ KV	1.63	1.50-1.85	.158	4	1.83	1.50-2.47	.287	9	.136
3/KV as % SVL	2.61	2.14-3.73	.753	4	2.34	1.80-2.87	.334	9	.139
Isthmus Width	1.20	1.0-1.5	.238	4	1.56	1.22-2.18	.345	9	.038
I W as % SVL	1.89	1.43-2.49	.441	4	1.97	1.65-2.35	.260	9	.390
Caudal Annuli	16.1	16-16.5	.250	4	16.7	15-19	1.09	9	.314
Knob Scales	44.3	40-49	4.43	4	46.0	43-48	1.73	9	.311
Forelimb L	27.9	16.2-32.8	4.99	14	31.3	23.4-36.3	3.67	14	.174
FL as % SVL	39.7	37.4-43.4	1.60	14	38.7	34.8-42.9	2.75	14	.286
Hindlimb L	34.3	20.3-39.6	6.03	14	37.0	27.1-43.3	4.36	14	.362
HL as % SVL	48.9	46.5-55.2	2.17	14	45.8	38.5-51.6	3.78	14	.059
Subdigital Lamellae	NA								
Latitudinal Range (m+f)		21.8-29.0°S							
Longitudinal Range (m+f)		115.6-119.6°E							

Table A1.10 Morphometric Data Analysis For *U. milii*

	MALES				FEMALES				
CHARACTER	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Collection Dates		8.6-5.4		41		7.3-5.5		49	
SVL	68.4	37.5-95.0	17.4	67	72.7	41.0-110	17.8	64	.117
Tail Length	45.7	23.0-73.2	15.6	45	46.1	24.5-63.9	11.2	35	.452
Total Length	105	51.0-158	30.8	67	104	43.0-152	29.4	62	NA
Tail as % SVL	69.6	59.3-83.3	8.13	45	67.1	55.778.8	6.00	35	.005
Head Width	15.6	9.3-23.1	3.91	57	15.6	9.3-23.1	3.62	62	.499
HW as % SVL	22.8	17.5-25.8	1.49	57	21.9	18.1-25.7	1.46	62	.00061
Head Height	8.78	4.8-13.0	1.95	32	8.59	5.1-11.5	1.63	47	.243
HH as % SVL	12.4	10.2-14.3	1.03	32	11.6	9.32-14.9	1.04	47	.00037
Head Length	20.0	15.7-26.7	3.09	10	18.4	12.5-23.0	2.75	15	.069
HL as % SVL	27.3	26.2-28.9	.894	10	26.4	23.9-30.9	1.76	15	.139
Tail Width	12.6	5.3-19.7	4.64	19	11.1	5.56-19.0	3.73	32	.072
TW as % SVL	18.2	12.3-25.7	3.31	19	15.4	9.52-23.6	3.33	32	.0016
Tail Height	6.93	3.20-12.0	2.47	19	6.48	3.55-9.80	1.69	32	.150
TH as % SVL	10.0	7.90-15.8	1.78	19	9.09	5.94-12.1	1.57	32	.017
Tail Volume	1463	124-3186	1085	19	1014	136-2868	726	31	.020
3/TV	10.4	4.99-14.7	3.46	19	9.49	5.14-14.21	2.40	31	,102
3/TV as % SVL	15.0	11.6-19.4	1.92	19	13.4	10.2-17.8	1.78	31	.0013
Peduncle	28.3	13.5-40.8	9.97	18	27.1	13.0-37.8	6.93	26	.280
Ped as % SVL	40.6	19.7-49.7	6.99	18	37.9	16.8-48.9	6.56	26	.117
Ear Height	3.05	2.33-4.41	.638	10	2.74	2.08-3.41	.379	15	.086
EH as % SVL	4.15	3.51-4.78	.410	10	3.96	3.11-4.94	.557	15	.370
Eye Diameter	4.98	3.80-5.77	.592	10	4.99	3.10-6.18	.796	15	.437
ED as % SVL	6.84	6.25-7.60	.446	10	7.15	6.06-9.07	.795	15	.088
Post Rostrals	6.61	5-8	.818	57	6.10	5-9	1.05	59	.0068
Post Mentals	5.68	2-8	1.39	56	5.71	3-8	1.22	59	.875
Transtubercular W	8.41	3.5-12.9	3.05	50	7.01	2.6-11.8	2.17	62	.0018
TtW as % SVL	11.9	8.51-16.4	2.16	50	9.61	6.19-12.7	1.42	62	<.0001
Transiliac W	7.75	6.59-9.38	.979	10	7.98	5.03-10.9	1.54	15	.397
TiW as % SVL	10.6	9.54-11.6	.676	10	11.3	10.1-12.8	.870	15	.015
Spur Tubercles	10.3	4-15	2.95	10	7.93	3-12	2.24	14	.029
Body Width	16.9	11.2-21.6	3.74	10	16.3	6.79-22.3	3.86	15	.233
BW as % SVL	22.4	15.1-27.7	4.22	10	22.9	16.1-28.7	3.34	15	.454
Fat Body	4.53	3-6	.915	15	4.12	1-6	1.13	25	.119
Lt Testis Length	4.64	0.8-9.0	2.30	41					
L T Width	2.78	0.4-6.2	1.67	41					
L T Depth	1.99	0.2-4.5	1.32	41					
L T Volume	25.9	.042-132	32.1	41					
Rt Testis Length	4.96	1.0-9.1	2.43	40					
R T Width	3.18	0.4-6.6	1.83	40					
R T Depth	1.90	0.2-4.5	1.16	40					
R T Volume	28.3	.052-133	32.9	40					
Rt+Lt Tvol	53.6	.094-264	62.2	41					
Lt Follicle 1					5.04	.250-23.4	6.28	46	
L F 2					2.13	.460-4.26	1.06	12	
L F 3					1.78	1.08-3.10	.602	11	
Rt Follicle 1					4.86	.150-23.5	5.68	46	
R F 2					2.13	.290-3.41	.956	11	
R F 3					1.54	.170-2.51	.537	11	
Lt+Rt F Diameter					9.90	.400-46.9	11.7	46	
L+R F D as %SVL					12.2	.952-65.3	13.5	46	

Table A1.10 (Continued).

U. MILII									
	MALES				FEMALES				
CHARACTER	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Rostral Width	3.49	2.67-3.97	.458	12	3.26	2.16-4.24	.576	15	.102
RW as % SVL	4.66	4.12-5.09	.320	12	4.63	3.95-5.13	.359	15	.313
R Height	1.51	1.14-2.03	.238	12	1.39	0.88-1.86	.220	15	.068
R H as % SVL	2.04	1.90-2.20	.107	12	1.99	1.59-2.47	0.24	15	.259
Mental Width	3.01	2.24-3.78	.436	11	2.65	1.66-3.60	.595	15	.042
MW as % SVL	3.97	3.46-4.45	.267	11	3.74	2.97-4.39	.449	15	.090
M Height	1.99	1.66-2.36	.265	11	1.73	1.09-2.30	.343	15	.019
MH as % SVL	2.61	2.47-2.87	.132	11	2.45	1.89-2.90	.244	15	.029
R + M Area	10.9	3.04-16.7	3.71	11	9.38	3.79-13.6	3.05	15	.098
/(RMA)	3.42	2.79-4.08	.402	11	3.02	1.95-3.69	.523	15	.024
/(RMA) as % SVL	4.47	4.30-4.69	.115	11	4.29	3.56	4.86	15	.042
Internasals	9.24	7-11	1.44	17	8.22	7-10	1.06	18	.031
Interorbitals	12.1	11-14	1.22	17	11.8	8-19	2.68	21	.558
Nasolabials	1	1	0	20	1	1	0	20	1
Perinasals	13.3	12-15	1.11	14	13.6	12-16	1.22	22	.449
Nasooculars	24.9	21-28	2.28	11	24.7	22-30	2.26	15	.786
Supralabials	10.7	10-12	.749	23	11.1	9-13	.997	27	.338
Infralabials	10.1	9-12	.912	20	10.3	9-12	.875	26	.450
Transpalpebral	8.67	7-11	.934	12	8.44	6-10	1.15	18	.367
Palpebral Edge	21.2	18-25	1.94	10	23.6	20-26	1.68	15	.0027
Midbody Scales	246	199-277	24.8	10	254	223-277	15.7	12	.334
Rostral-Pelvis Sc	458	430-494	26.0	10	459	403-494	26.5	12	.649
Mental-Vent Sc	301	269-345	20.7	10	328	304-382	21.2	11	.015
Rosette Scales	11.6	10-13	.843	11	12.2	11-13	.651	12	NA
Inter-R Scales		1-8		10		1-7		13	NA
Knob Characters	NA	NA	NA	NA	NA	NA	NA	NA	NA
Isthmus Width	NA	NA	NA	NA	NA	NA	NA	NA	NA
Caudal Annuli	NA	NA	NA	NA	NA	NA	NA	NA	NA
SDL Manus 1	11.6	10-13	0.97	10	10.9	9-12	.77	15	.048
SDLM 2	14.9	12-16	1.29	10	14.2	12-16	1.12	15	.179
SDLM 3	17.8	16-20	1.32	10	16.6	15-18	.745	15	.012
SDLM 4	17.4	16-19	.966	10	16.6	15-18	.929	15	.066
SDLM 5	14.6	13-16	.966	10	13.4	12-16	1.22	15	.020
SDLM Total	76.3	73-83	3.27	10	71.8	67-75	2.97	15	.0019
SDL Pes 1	10.9	9-12	1.10	10	10.7	10-12	.825	15	.641
SDLP2	15.0	12-16	1.25	10	14.6	13-16	.842	15	.410
SDLP 3	19.3	16-21	1.33	10	19.1	15-21	1.49	15	.703
SDLP 4	20.0	19-21	.816	10	19.4	17-21	1.34	15	.192
SDLP 5	20.7	18-23	1.57	10	19.0	16-21	1.36	15	.0096
SDLP Total	85.9	80-91	2.81	10	82.8	76-89	4.28	15	.057
Forelimb L	28.0	23.1-32.9	3.04	11	25.4	16.7-31.2	4.22	15	.039
FL as % SVL	38.0	34.4-43.2	2.63	11	36.3	30.3-41.6	3.29	15	.132
Hindlimb	34.1	28.0-41.8	4.10	11	30.1	19.6-38.3	5.38	15	.014
HL as % SVL	46.2	43.2-49.5	1.92	11	42.9	33.8-48.0	3.67	15	.0050
Latitudinal Range (m+f)		24.8-37.1°S							
Longitudinal Range (m+f)		113.3-152.1°E							

Table A1.11 Morphometric Data Analysis For *U. sphyrurus*

	MALES				FEMALES				
CHARACTER	MEAN	RANGE	STD	N	MEAN	RANGE	STD	N	*T*-TEST
Dates		8.5-5.7		6		6.3-11.5		5	
SVL	62.0	38-73	11.3	13	70.1	51-85	9.84	13	.032
Tail Length	29.7	17.8-38	7.11	9	36.2	29.2-40.0	3.64	10	.010
Total Length	86.0	55.8-105	14.7	13	101	80.2-123	14.0	13	NA
Tail as % SVL	50.5	41.1-64.4	7.04	9	52.8	47.7-57.3	3.80	10	.188
Head Width	13.4	7.57-16.0	2.57	13	15.3	12.6-21.2	2.44	13	.032
HW as % SVL	21.6	19.3-24.6	1.43	13	22.0	17.4-29.0	3.15	13	.334
Head Height	7.56	4.67-9.23	1.44	10	8.33	7.42-9.64	.668	11	.065
HH as % SVL	12.4	11.0-14.1	.901	10	12.3	10.4-15.1	1.20	11	.362
Head Length	16.7	1.2-19.5	2.99	10	18.4	15.6-21.1	1.69	12	.057
HL as % SVL	27.5	23.6-30.9	1.96	10	26.8	24.7-30.6	1.71	12	.202
Tail Width	11.0	4.4-15.6	3.77	8	13.6	8.78-19.2	3.12	10	.267
TW as % SVL	18.4	11.6-23.2	4.33	8	19.7	14.4-24.7	3.53	10	.031
Tail Height	5.98	2.94-8.91	2.29	8	7.38	5.65-9.28	1.10	10	.270
TH as % SVL	9.92	6.19-12.7	2.39	8	10.8	8.68-12.9	1.45	10	.077
Tail Volume	642.9	60.3-1266	454	8	998	413-1847	433	10	.362
3/TV	8.04	3.92-10.8	2.48	8	9.8	7.45-12.3	1.45	10	.308
3/TV as % SVL	13.4	10.1-16.4	2.45	8	14.3	11.9-16.8	1.59	10	.039
Peduncle	10.2	7.25-13.9	2.18	8	13.1	10.7-17.1	2.23	10	.098
Ped. as % SVL	17.6	12.9-23.5	3.38	8	19.1	16.8-22.2	1.76	10	.110
Ear Height	2.19	1.45-3.04	.490	10	2.41	1.96-3.07	.370	12	.318
EH as % SVL	3.62	2.82-4.71	.610	10	3.52	2.80-4.51	.47	12	.219
Eye Diameter	4.24	2.60-5.33	.857	10	4.66	3.80-5.36	.515	12	.332
ED as % SVL	6.98	5.82-7.86	.730	10	6.81	5.97-7.82	.590	12	.066
Post Rostrals	6.62	6-8	.650	13	6.67	5-8	.778	12	.692
Post Mentals	3.38	2-5	.870	13	3.58	3-5	.669	12	.485
Transtubercular W	8.16	3.46-10.8	2.41	13	7.24	5.24-10.1	1.35	13	.033
TtW as % SVL	12.9	9.11-16.1	2.30	13	10.3	8.59-11.9	1.15	13	.00019
Transiliac Width	6.55	3.87-8.57	1.48	10	7.38	6.02-8.17	.733	12	.320
TiW as % SVL	10.5	8.92-12.0	.990	9	10.8	8.60-12.1	1.07	12	.174
Spur Tubercles	15.2	3-19	6.83	5	12.7	6-19	4.01	12	.084
Body Width	11.9	7.03-15.4	2.92	10	13.6	7.57-21.7	3.92	12	.443
BW as % SVL	19.3	15.9-21.6	2.08	10	19.9	10.0-26.8	5.39	12	.326
Fat Body	4.0	1-6	1.41	8	3.42	2-6	1.38	12	.212
Lt Testis Length	5.78	1.22-10.0	2.82	12					
L T Width	2.75	0.57-4.50	1.30	12					
L T Depth	1.96	0.33-3.15	1.02	12					
L T Volume	37.0	0.23-74.0	30.0	12					
Rt Testis Length	4.88	1.25-10.3	2.43	12					
R T Width	2.99	0.30-5.50	1.57	12					
R T Depth	1.74	0.16-3.15	0.90	12					
R T Volume	18.4	.031-42.4	16.0	12					
Lt+Rt TVol	44.0	.203-89.9	35.1	12					
Lt Follicle 1					4.63	0.5-11.4	3.73	13	
L F 2					3.13	2.61-3.64		2	
L F 3					2.21	2.03-2.38		2	
Rt Follicle 1					4.51	0.50-11.9	3.95	13	
R F 2					3.50	3.18-3.82		2	
R F 3					2.86	2.80-2.91		2	
Lt+Rt FD					9.14	1.00-23.3	7.65	13	
L+R FD %SVL					12.6	1.49-23.8	10.1	13	

Table A1.11 (Continued).

U. SPHYRURUS									
CHARACTER	MALES				FEMALES				*T*-TEST
Rostral Width	3.23	2.32-3.86	.536	9	3.52	3.09-3.87	.264	12	.061
RW as % SVL	5.46	4.53-6.46	.588	9	5.15	4.59-6.12	.410	12	.084
R Height	1.28	0.79-1.55	.263	9	1.37	1.06-1.53	.131	12	.156
RH as % SVL	2.15	1.77-2.63	.261	9	2.01	1.75-2.19	.171	12	.069
Mental Width	2.63	1.89-3.19	.448	9	3.00	2.58-4.41	.512	12	.050
MW as % SVL	4.44	3.73-4.97	.434	9	4.38	3.64-6.04	.648	12	.412
M Height	1.75	1.13-2.15	.295	9	1.82	1.52-2.35	.236	12	.284
MH as % SVL	2.96	2.51-4.10	.463	9	2.65	2.17-3.14	.301	12	.039
R + M Area	21.6	3.91-40.7	11.6	9	27.1	13.7-43.7	9.66	12	.123
/(RMA)	4.46	1.98-6.38	1.39	9	5.13	3.70-6.61	.917	12	.097
/(RMA) as % SVL	7.31	5.21-9.12	1.27	9	7.43	6.72-9.05	.683	12	.386
Internasals	9.22	8-11	1.09	9	8.75	7-10	0.87	12	.282
Interorbitals	16.1	15-19	1.45	9	15.1	13-17	1.14	12	.094
Nasolabials	1	1	0.00	18	1	1	0	24	1
Perinasals	10.4	9-12	1.13	7	10.2	9-12	1.03	10	.672
Nasooculars	18.1	16-20	1.81	8	16.4	14-19	1.80	11	.051
Supralabials	12.7	11-15	1.33	14	13.3	11-16	1.17	20	.266
Infralabials	11.9	11-15	.666	14	11.4	9-14	1.51	20	.341
Transpalpebral	5.2	4-6	.837	5	5.29	4-6	.756	7	.428
Palpebral Edge	21.8	18-25	2.86	5	19.4	17-22	1.90	7	.057
Midbody Scales	192	175-204	11.3	5	187	177-194	6.09	6	.184
Rostral-Pelvis Scales	378	358-397	18.3	4	379	363-401	13.5	6	.929
Mental-Vent Scales	296	270-314	20.7	5	288	258-318	23.6	6	.560
Rosette Scales	12	12		5	12.2	12-13		6	NA
Inter-R Scales		0-4		4		0-3		5	NA
Knob Characters	NA	NA	NA	NA	NA	NA	NA	NA	NA
Isthmus Width	NA	NA	NA	NA	NA	NA	NA	NA	NA
Caudal Annuli	NA	NA	NA	NA	NA	NA	NA	NA	NA
SDL Manus1	9.71	8-11	1.11	9	9.57	8-11	.976	9	.803
SDL M 2	14.14	13-15	0.90	9	13.3	12-15	.951	9	.109
SDL M 3	15.6	14-17	1.27	9	14.7	13-17	1.50	9	.271
SDL M 4	16.3	15-17	,756	9	15.3	14-18	1.38	9	.119
SDL M 5	13.43	11-16	1.62	9	12.3	11-14	.951	9	.133
SDL M Total	69.1	62-73	3.98	9	65.1	61-74	4.81	9	.116
SDL Pes 1	9.57	8-11	.976	9	8.71	8-9	.488	9	.060
SDL P 2	14.3	13-15	.756	9	13.3	12-15	1.25	9	.096
SDL P 3	18.1	17-19	.690	9	16.0	15-18	1.00	9	.0005
SDL P 4	18.4	16-20	1.51	9	16.6	15-18	0.976	9	.018
SDL P 5	18.4	16-20	1.27	9	16.4	10-21	3.26	9	.156
SDL Pes Total	78.9	73-82	3.81	9	71.0	63-80	5.20	9	.002
Forelimb Length	24.9	14.9-29.4	5.51	10	27.2	22.6-31.1	2.74	12	.108
FL as % SVL	40.6	36.3-48.1	3.19	10	39.7	35.9-44.3	2.63	12	.247
Hindlimb	30.8	16.9-37.7	7.08	10	33.3	25.5-38.4	4.30	12	.159
HL as % SVL	50.2	44.5-60.2	4.52	10	48.5	41.8-53.4	3.39	12	.170
Latitudinal Range (m+f)		28.1-32.2°S							
Longitudinal Range (m+f)		151.1-152.0°E							

Appendix 2

2.1 List Of *Nephrurus* And *Underwoodisaurus* Specimens Examined

Abbreviation	Museum/Collection name
AM	Australian Museum, Sydney.
ANWC	Australian National Wildlife Collection, Canberra.
BMNH	British Museum of Natural History, London.
MANT	Museum and Art Galleries of the Northern Territory, Darwin.
QM	Queensland Museum, Brisbane.
SAM	South Australian Museum, Adelaide.
TA	Personal collection or personal observation.
TPZ	Taronga Park Zoo.
VM	Victorian Museum, Melbourne.
WAM	Western Australian Museum, Perth.

List of Specimens:

This list includes species name, number of specimens examined for each species (N), collection names and specimen registration numbers. Where more than one specimen has been assigned to a specific museum registration number each specimen has been given that number plus a suffix a, b, c, ...etc. Museum specimens without collection data are prefixed by the letters ND (no data). Gecko embryos and geckos of other genera are not included. A total of 1210 specimens are listed. Registration numbers prefixed by TAL represent repeat (longitudinal study) observations during development of live specimens (less than two percent of total). Live specimens were obtained under licences from New South Wales, Northern Territory, Queensland, South Australia and Western Australia licensing authorities.

Nephrurus amyae (N = 29)

AM

R10371, R11965, R20449, R49716, R50542, R90198, R104458, R141711.

MANT

R2458, R5466, R5969, R12380, R14991, R33715, R33717, R33720, R33722, R33724, R33726, R33922.

TA

TA4, TA5, TA1575, TA1576, TA1761, TA1762, TA1763, TA1765, TA1820.

Nephrurus asper (N = 88)

AM

R1883, R4923, R14183, R15107, R20094, R31773, R40070, R42760, R55786, R63065, R83250, R83255, R83256, R83257, R83258, R102102, R102546, R102547, R102599, R102600, R102601, R102603, R102604, R102605, R102607, R102608, R102610, R105644, R105700, R105762, R107165, R107703, R110544, R110562, R113116, R113264, R113265, R113266, R113270, R113271, R113273, R113852, R114829, R117811, R117812, R120094, R128183, R125387, R128183, R128796, R130721, R130722, R130726, R128796, R141711, R141711, R142962, R160722, ND1.

BMNH

1946.8.23.34.

MANT

R266, R1144.

QM

J738, J2125, J3443, J4960, J9132, J9912, J15565, J22179, J24921, J28699, J31545, J31976, J34205, J35040, J40070, J40165, J44689, J44948, J52872, J53650, J54644, J57652, JI57993, J58850, J97993.

TA

TA2, TA3, TA1295, TA1377, TA1378, TA1500, TA1530, TA1531, TA1533, TA1534, TA1577, TA1595, TA1674, TA1675.

N. deleani (N = 16)

SAM

R20213, R21862, R21863, R38599.

TA

TA1666, TA1667, TA1668, TA1669, TA1670, TA1672, TA1697, TA1772, TA1773, TA1774, TA1775, TA1776.

N. laevissimus (N = 305)

AM

R7193, R7693, R10282, R15287, R27936, R31593, R31774, R32397, R32909, R40457, R41674, R49080, R49081, R49529, R49581, R49672, R49680, R49720, R49730, R49736, R49750, R65100, R66651, R70039, R70049, R70050, R73915, R76644, R86506, R86507, R91077, R91078, R91079, R91080, R91081, R100910, R101551, R101552, R101580, R101785, R101786, R101981, R102151, R102446, R102447, R102448, R102449, R102450, R102452, R102453, R102454, R102457, R102458, R102519, R125160, R125460, R128420, R128425, 128426, R128427, R128429, R128664, R128671, R128673, R128674, R128675, R128676, R128677, R128678, R128679, R128680, R161787.

WAM

R8416, R8417, R12223, R12226, R12230, R14843, R14844, R14845, R14943, R26385, R27027, R30844, R30845, R30846, R36713, R39137, R40229, R40918, R40919, R40920, R40921, R40922, R40928, R40929, R40930, R40937, R40938, R40945, R40946, R42312, R46637, R48647, R48648, R48650, R48651, R48677, R48678, R48679, R48739, R48760, R48761, R48762, R48763, R51955, R53545, R53546, R53547, R53567, R53568, R53569, R53570, R53571, R53572, R53573, R53659, R54531, R58703, R58715, R63436, R63441, R63442, R63443, R63511, R63512, R63513, R63514, R63709, R63715, R63725, R63744, R63759, R63888, R63889, R63956, R63983, R64012, R64015, R64115, R64116, R64129, R64147, R64150, R64151, R64187, R64235, R64249, R64250, R64251, R64261, R65639, R65680, R65681, R65687, R65688, R65690, R65695, R65723, R65724, R65740, R65741, R65742, R65754, R65755, R65781, R65782, R65783, R68042, R69912, R69913, R69914, R72620, R72698, R72722, R72736, R72738, R72739, R75790, R81023, R86001, R86002, R86003, R86004, R86005, R86006, R86007, R86008, R86009, R86010, R86010, R86011, R86012, R86013, R86014, R86015, R86016, R86017, R86018, R86019, R86020, R86021, R86022, R86023, R86024, R86025, R86026, R86027, R86028, R86029, R86030, R86031,

R86032, R86033, R86034, R86035, R86036, R86037, R86038, R86039, R86040, R86041, R86042, R86043, R86044, R86045, R86046, R86047, R86048, R86049, R86050, R86051, R86052, R86053, R86054, R86055, R86056, R86057, R86058, R86059, R86060, R86061, R86062, R86063, R86064, R86065, R86066, R86067, R86068, R86069, R86070, R86071, R96036.

MANT

R1713, R11006, R12490.

QM

SAM

R665, SNP1118, R14053, R14987b, R14987d, R15497, R15566a, R16761, R17462, R18221, R18222, R18284, R21366, R25558, R25560, R26379, R31835, R31860, R32053, R32200, R32213, R33044, R32195, R32232, R32247, R32248, R32250, R32251, R33994, R41866, R42525, R42526, R42553, R42556, R42584, R42544, R42586.

TA

TA1776, TA1777.

N. levis (N =234)

AM

R2039, R2407, R5397, R6111, R6754, R6917, R7672, R10906, R11966, R11967, R13028, R13265, R13941, R13942, R16471, R17741, R17529, R17585, R20845, R21217, R31594, R31594, R31648, R40501, R47529, R47718, R49081, R49085, R49091, R49673, R49676, R49681, R49692, R49693, R49718, R49719, R49725, R50666, R50674, R50675, R52141, R41674, R31594, R50676, R52140, R55787, R55992, R56949, R57074, R57097, R57097, R57198, R60282, R65100, R65287, R66651, R70039, R70049, R70050, R73915, R76644, R83251, R83253, R83254, R83259, R83260, R83261, R83262, R83263, R83264, R83265, R83267, R83268, R83270, R83271, R86499, R92369, R93002, R93272, R95426, R96112, R96113, R96126, R96130, R96131, R96160, R101552, R101552, R101785, R101981, R101981, R102448, R102449, R102450, R102451, R102451, R102457, R102457, R102458, R102458, R105641, R105700, R105729, R105729, R110278, R110594, R110594, R113264, R113266, R113267, R113268, R113270, R113271, R113272, R113273, R114550, R114829, R114830, R117811, R117812, R118594, R118594, R118595, R118596, R125151, R125152, R125153, R125154, R125155, R125156,

R125157, R125158, R125159, R128128, R128128, R128248, R128643, R128782, R132988, R134737, R134742, R138436, R138437, R140502, R140503, R140504, R140555, R140556, R140557, R140558, R140559, R140560, R140561, R140562, R141971, R141972, R142988.

BMNH

1889.5.13.1, 1946.8.23.42.

MANT

R196, R1491, R15142, R15180, R1607, R17626, R1765, R1963, R7561, R18170.

SAM

R152, R707, R878, R5443, R5503, R7559, R7560, R7563, R7566, R9010, R1884a, R1962a,

TA

TA296, TA311, TA1510, TA1559, TA1571, TA1572, TA1573, TA1574, TA1599, TA1600, TA1641, TA1648, TA1674, TA1675, TA1693, TA1694, TA1695, TA1697, TA1725, TA1726, TA1727, TA1728, TA1729, TA1730, TA1731, TA1732, TA1733, TA1734, TA1735, TA1736, TA1737, TA1750, TA1751, TA1752, TA1753, TA1754, TA1755, TA1756, TA1757, TA1758, TA1759, TA1760, TA1764, TA1765, TA1781, TA1782, TA1783, TA1784, TA1785, TA1786, TA1787, TA1822, TA1823.

N. sheai (N = 41)

AM

R12876, R13403, R64731, R70029, R70551, R70552, R70562, R72980, R73903, R73904, R77269, R88668, R93181, R93182, R110544, R13403, R140279.

MANT

R297, R2377, R3760, R4027, R11464, R12493, R13485.

WAM

R64731, R70029, R70551, R70552, R70562, R72269, R73903, R73904, R77269, R77585, R83359, R83531, R83532, R86927, R87082, R87092.

TA

TA1831.

N. stellatus (N = 73)

SAM

R641, R8392, R12614a, R12614b, R12614c, R14504a, R14504b, R14561a, R14561b, R14561c, R15205a, R15205a, R17663, R17954, R18515, R18516, R19597, R19598, R19599, R20773, R20791, R20792, R20799, R20800, R20809, R23785, R24811, R25344, R25345,R25346, R28438, R28439, R29839, R31898, R32300, R36552, R36563, R36573, R36615, R36616, R36617, R36648, R36650, R36651, R36652, R36653, R36659, R36661, R36686, R39176.

TA

TAL1676, TAL1676, TA1677, TA1766, TA1767, TA1768, TA1769, TA1770, TA1771, TA1789, TA1790, TA1791, TA1792, TA1793, TA1794, TA1795, TA1796, TA1797, TA1798, TA1799, TA1800, TA1801, TA1802.

N. vertebralis (N = 61)

WAM

R222, R1899, R4485, R4899, R5300, R13112, R17110, R17936, R29494, R34040, R34041, R34042, R37774, R37775, R48031, R48032, R48712, R49032, R49085, R49242, R49242, R49242, R53023, R53548, R53574, R59018, R65964, R65984, R69049, R69081, R69105, R69106, R69133, R69228, R70120, R72812, R73418, R74717, R74718, R74718, R74719, R75577, R76131, R77990, R78156, R78520, R78597, R78640, R84112, R84433, R87405, R87406, R87415, R87840, R87841, R96161, R96162, R100897, R100899, R101488.

TA

TA1832.

N. wheeleri (N = 34)

AM

R100597, R100897, R100899

BMNH

1946.8.23.52.

WAM

R733, R1168, R1396, R6417, R16528, R19092, R22628, R2297736, R26751, R73160,

R74883, R74884, R74885, R82594, R82595, R82596, R84113, R84114, R84115, R89954, R97736, R97776, R102163, R114106, R114268, R114296, R114628, R117050, R125024.

TA

TA1614.

U. milii (N = 304)

AM

R1047, R2163, R2426, R2465, R2481, R2691, R2951, R3115, R3412, R3427, R3435, R3595, R3868, R3875, R4412, R4566a, R4566b, R4584, R4585, R4923, R4924, R5293, R5310, R6089, R6090, R6116, R6259, R7144, R7177a, R7177b, R7177c, R7670, R7671, R7727, R8375, R8376, R9136, R9448, R10033, R10117, R10465, R10550, R10986, R11149, R11718a, R11718b, R12205, R12206, R12577, R13128, R12413, R14642, R14993, R14994, R20353, R20534, R20561, R26031, R26187, R26584, R26585, R27327, R27328, R27348, R27799, R28066, R28546, R29702, R29703, R39505, R39506, R40118, R40422, R41201, R42716, R44721, R45330, R45336, R45350, R45351, R45352, R45353, R50672, R50673, R54079, R54080, R55812, R55813, R55814, R55816, R55818, R61518, R61519, R61520, R61521, R64942, R67656, R67657, R67658, R69205, R69206, R69718, R69810, R69841, R69842, R69844, R69845, R69848, R69851, R69853, R69854, R69856, R69857, R69858, R69860, R70038, R70042, R70130, R76711, R76712, R76713, R81540, R81541, R81542, R81763, R86212, R86213, R86332, R86335, R86336, R86337, R89140, R89220, R93422, R93815, R93922, R93926, R93929, R93930, R93931, R94644, R94833, R94837, R95851, R97919, R99367, R101967, R101968, R102611, R102612, R102615, R102616, R102617, R102618, R102619, R103560, R103717, R104822, R105614, R106613, R106614, R106940, R107697, R107914, R107919, R107922, R108909, R114479, R114742, R115667, R115714, R115740, R115741, R115742, R115743, R115788, R118605, R118606, R123948, R123972, R125161, R125162, R125163, R125164, R125165, R123948, R127874, R128378, R128394, R128395, R128459, R128540, R129323, R129328, R130060, R130221, R130985, R133664, R133977, R133978, R133979, R134564, R142799, R142780, R142781, R142783, R145422, ND1, ND2, ND6.

MANT

R5028, R9213, R15281, R19264, R34320.

QM

J50338, J50339, J50340, J56054, J56909, J58887.

TA

TAb303, TAb304, TAb305, TAb306, TAb307, TAb308, TAb309, TAb310, TAb311, TAb312, TAb313, TA14, TA63, TA68, TA131, TA207, TA260, TA430, TA699, TA700, TA701, TA721, TA735, TA736, TA745, TA806, TA1143, TA1214, TA1215, TA1259, TA1260, TA1334, TA1335, TA1345, TA1346, TA1430, TA1431, TA1444, TA1446, TA1447, TA1448, TA1463, TA1464, TA1465, TA1480, TA1512, TA1513, TA1518, TA1519, TA1528, TA1560, TA1561, TA1578, TA1596, TA1597, TA1606, TA1607, TA1608, TA1642, TA1643, TA1649, TA1650, TA1708, TA1709, TA1710, TA1711, TA1712, TA1713, TA1714, TA1745, TA1746, TA1747, TA1748, TA1800, TA1802, TA1804, TA1806, TA1808, TA1810, TA1812, TA1814, TA1816, TA1984.

U. sphyrurus (N = 25)

AM

R10332, R106935, R125388, R140837, R140838, R141562, R15195, R15642, R1818, R35188, R4880, R51688, R5617, R6770, R69717,

TA

TA1700, TA1701, TA1702, TA1703, TA1704, TA1738, TA1739, TA1740, TA1837, TA 1838,

Appendix 3

3.1 Observations and Experiences with Husbandry and Pathology of *Nephrurus* and *Underwoodisaurus* Geckos

A3.1 Husbandry

As far as practicable a natural environment was provided for the geckos (but without the extremes which might occur in nature) with diurnal and annual temperature cycling, thermal and illumination gradients, as well as controlled or natural photoperiods, increased subterranean water potentials and suitable refugia. The nutritional requirements were met with a variety of arthropods and a constant supply of water (Annable, 1992: Appendix 3). The major object of husbandry was to approach an optimum breeding environment so that more eggs would be produced and they would be normal and healthy. Details of egg incubation are provided in Chapter 4.

The general requirements for gecko husbandry and captive breeding have been outlined (Zimmerman, 1986; Obst et al., 1988; Seufer, 1991; Henkel and Schmidt, 1995) and also for *Nephrurus* species (Sameit, 1990; Annable, 1992; Henkel and Schmidt, 1995; Wagner and Lazik, 1996). The majority of specimens used in this research was housed in rectangular glass or methyl methacrylate vivaria from about 450mm to 1000mm long. Most vivaria were kept at room temperature with an approximately natural photoperiod at 33°05'S, 151°26'. Two vivaria were given set photoperiods using timer clocks. Three vivaria were given a temperature gradient by using 40 watt or 60 watt lighting at one end or side of the vivarium. Four vivaria were provided with a low wattage Thermofilm 90mm wide heat strip along one side of the vivarium. Two of these were left switched on permanently (for tropical species) and the other two were connected to the photoperiod timer, remaining switched on during the day-time only. The photoperiod time-clocks were maintained at approximately 13 hours in the summer and 12 hours in the winter. All except one of the vivaria were supplied with fine sand as a substrate, using red sand from central Australia where possible for the *Nephrurus* species. This sand is very fine, has a high iron content and tends to retain moisture and to remain solid after moistening so that geckos

could dig their own burrows even after the sand has dried out. Certain grades of fine builder's sand have similar properties but generally have a lower iron and other salts content and it is possible that the minerals present in the sand may be important in *Nephrurus* gecko metabolism. For *U. milii* a substrate of coarse washed river sand (with a lower iron content) was used. *U. sphyrurus* was the only species found not to tolerate these conditions. This species was found to do well with a dry stony substrate mixed with a very course vermiculite and small strips of paper bark (*Melaleuca* sp.) to simulate the leaf litter preference (even dead *Eucalyptus* leaves can be very toxic in an enclosed environment). For refugia, 210mm long by 40mm diameter cardboard tubes, partially flattened and closed at one end were used. All vivaria were provided with suitable bark, logs, rocks and/or plastic refugia.

Water was provided in deep glass petri-dishes or similar. As these geckos drink free water only occasionally it was found to be unnecessary to have a continuous water supply and dishes were refilled about once every two weeks. Many gecko breeders now recommend regular use of vitamin and mineral supplements (Seufer, 1991; Wagner and Lazik, 1996). I have not found this necessary for *Underwoodisaurus* species when using natural substrates and a varied natural diet. The provision of such a diet became impractical as the number and variety of geckos held increased. I do occasionally provide a vitamin and mineral supplement to any gecko that does not look in peak condition but also provide as wide a variety of arthropods as possible. Cultures of meal worms (Tenebrio molitor), crickets (*Gryllus* sp. and several Australian native species), wax moths (*Galleria* sp.), cockroaches (*Periplaneta* sp., *Blattella* sp. and the Australian native species *Nauphoeta cinerea*) and silverfish (*Lepisma saccharina*) have been maintained to provide dietary variation. In captivity both *N. levis* and *U. milii* species geckos are observed to feed on a wide range of arthropods and other small organisms. *U. milii* has been observed to feed on many species of grasshoppers (Orthoptera), numerous cricket species (gryllotalpids and others), certain cockroach species (Blattaria), certain beetles (Coleoptera) including mealworm larvae (*Tenebrio molitor*), but not spiky, very hard or brightly coloured species, silverfish (*Lepisma saccharina*), many moderate sized moth species and their larvae particularly the dull coloured greyish and brownish species such as grain moths (*Nemapogon granella*), wax moths (*Galleria mellonella*), grass tree moths (*Meyriccia latro*), army worms

(*Mythimna convecta*), fruit moths (Tortricidae spp.) and bogong moths (*Agrotis infusa*). Moths not eaten include the red, orange or yellow coloured species such as tiger moths (Arctiinae) including the common species *Argina astrea*. Interestingly they will eat a brown and mauve coloured hepialid moth species. Other species consumed include several species of mantids (praying mantis), phasmatids (stick insects), woodlice (*Armadillidium* and *Oniscus* spp.), pea crabs (Brachyura), most moderate sized species of spiders (Arachnida) including funnel-web spiders (*Atrax robustus*) but excluding red-back spiders (*Latrodectus mactans*), several scorpionids including *Urodachus manicatus*, *Lychas marmorius* and other species, centipedes (Chilopoda) (all four of the local species), small earthworms (*Lumbricus*? sp.), small skinks (*Lampropholis delicata*), small geckos and neonatal mice (Annable, 1992; Wegener and Lazic, 1996; and unpublished personal observations). The arthropods which are refused generally include those organisms which have an olfactory-aimed anti-predator defence mechanism such as many hemipterans, millipedes, certain cockroaches, certain caterpillars (spiny or brightly coloured), wasps, ants and red-back spiders. Some venomous arthropods such as scorpions, spiders and centipedes are eaten with impunity. The geckos are not noticeably affected by bites and stings from these arthropods. The negative response to red or orange coloured arthropods is not innate but learned by juveniles at their first attempt to eat such species when they demonstrate a 'nasty flavour' response consisting of stepping backwards, repeated licking of the snout, turning the head from side to side, wiping the snout on the substrate and possibly scratching at the snout with the forelimbs. The intensity of such an event is so impressive that they will refuse to attack another such prey species even after years without exposure indicating a significant long-term memory. Dead or stationary arthropods are not usually eaten but are occasionally, perhaps due to apparent relative movement when the gecko is moving past such a prey species. All *Nephrurus* species observed frequently lick dry sand off the substrate but fruit pulp etc. is not taken by *Nephrurus* or *Underwoodisaurus* species. Very slow moving creatures such as slugs, snails and planarians do not seem to trigger any feeding response (although they do in other reptiles such as *Hemisphaeriodon gerrardii*). Two specimens of *U. milii* have now been maintained under room temperature conditions for fifteen years and the fourth generation are now reaching maturity suggesting adequacy of husbandry conditions.

During this study an improved method of providing egg-laying facilities was developed as follows. A plastic box about 290 mm long x 140mm wide x 130mm deep and with a tight fitting lid was provided. An oval hole about 35mm wide was cut in the middle of one end, centred at about 80 mm from the base. The box was then filled two thirds full of damp (but not wet) red sand. A strip of Melaleuca bark covered about two thirds of the surface of the sand to help retain moisture and to provide cover under which the geckos are more likely to lay eggs. The lid was then replaced and the box buried in the sand at the edge of a vivarium away from the heat source to a depth such that a gecko walking around the perimeter would be likely to walk straight into the opening. It was found that when this 'nesting box' was provided the females almost invariably used it to lay their eggs in. One exception was *U. sphyrurus* which excavated a nesting site in coarse vermiculite substrate and laid its eggs directly on the base of the vivarium even though a nesting box containing a mixture (~50:50, volume: volume) of slightly moist fine vermiculite and sand was provided as above. A major advantage of this system is that it will remain damp enough for egg survival for several weeks before redampening of the sand is required and even then water was needed only near the entrance hole. One disadvantage was that with an opaque lid any disturbance of the sand due to egg laying may easily remain undetected but a transparent lid perhaps with an opaque flap would overcome this problem.

Captive male and female hatchlings and juveniles of *N. asper, N. levis*, and *U. milii* grow at almost identical rates when maintained together under similar conditions indicating that males mature earlier (at a smaller size). Females grow for longer to reach sexual maturity and continue to grow to a larger size in all 11 study species except *U. milii* (the largest specimen was a female in all species).

A3.2 Pathology in *Nephrurus* and *Underwoodisaurus* geckos

Pathology of these geckos has not been investigated in detail but a number of conditions have been noted during the course of this project. Parasites include the red *Geckobia* mites which are common in wild-caught specimens. These mites are commonly found firmly attached to areas of the skin where it is presumably thinner such as around the eyes and ears and in creases on the body or limbs. Less commonly they are found on more exposed

surfaces such as the dorsum or tail. The majority of specimens found in the wild have no mites. Ticks also occur but are uncommon on these geckos. One preserved specimen of *N. levis* was found to have the head of an ant firmly clamped to the gecko's neck by the jaws.

A number of geckos have been found with parasitic nematodes in the intestines or in body cavities or coiled up under the skin. A small cestode has also been found on occasion under the skin. Although blood parasites are sometimes found in reptiles (Lane and Mader, 1996; Campbell, 1996) none were found in the three blood smears taken from *N. levis* and *U. milii*. Behaviour during sickness is variable depending on the pathology involved.
A *Cryptosporidium* was seen in a faecal smear taken from a sick looking *N. levis* specimen but it is not known if this organism was the primary cause of the problem.
A post-mortem examination of an apparently healthy *N. asper* that died after a few days with loss of appetite showed no obvious visceral defects. A swab from the mouth plated out on McConkey's medium showed gram positive bacilli. A throat culture showed lactose using coliforms and some gram positive cocci possibly *Staphylococcus*. A swab from the peritoneum showed some enteric gram negative rods and also lactose using coliforms. Using malachite green, spores were shown on the bacilli from the throat swab probably indicating *Bacillus subtilis* or *B. cereus* which are known to produce toxic effects (Pelczar et al., 1993).

Abnormal conditions and behaviours observed included:

1. Twitching of the head associated with a presumed infection.
2. Basking under an incandescent lamp also associated with a presumed infection.
3. Loss of appetite may be related to a sudden decrease in temperature.
4. Sluggish movements associated with aging or presumed infection.
5. Loss of alertness, cause unknown.
6. Anaemia as shown by very pale oral mucous membranes.
7. Rickets (calcium deficiency) shown by swollen limb joints, deformed scapulae and abnormal mobility.
8. Incomplete sloughing associated with injury or dietary deficiency.
9. Regurgitation of food possibly because of inappropriateness of food.

10. Diarrhoea associated with *Cryptosporidium* infection.
11. Failure to thrive of uncertain aetiology but possibly linked to inadequate thermal regime.
12. Tumours of bone and of soft tissues.
13. Sudden death, cause often unknown but may be due to fighting.

An additional and possibly very significant observation was made that only one egg out of ten apparently good eggs hatched where the gravid females (four *N. deleani* and one *U. sphyrurus*) had been transported by road over long distances. This observation suggests that prolonged vibration of the embryo prior to oviposition may be lethal to the embryo even where the gravid female is apparently unaffected.

Appendix 4

4.1 Measurements of *Nephrurus* and *Underwoodisaurus* Gonads

Measurements were made as described in Chapter 4 and Appendix 1.

1. Dimensions of testes (T). Museum specimens were dissected and the three perpendicular diameters (length, width and depth) of each testis were measured *in situ*. The 'length' was the greatest diameter of the testis (usually orientated along the long axis of the gecko). The 'width' was the largest width of the testis usually orientated in a near vertical plane. The 'depth' was the smallest diameter of the testis usually orientated in the transverse plane. From these measurements, values for testicular volume were calculated using a formula for the volume of a laterally compressed prolate spheroid *i.e.* with three different perpendicular ellipsoidal planes. $V = 4*\pi*r_1*r_2*r_3/3$. Where V = volume in mm^3, and r_1, r_2, and r_3 = testis radii of width, length and depth respectively in mm. Testes that were damaged (<1%) *e.g.*, by desiccation, trauma or disease, were not included in data tables. Testes removed for histological preparation were measured after orchidectomy. The cube root of the combined testicular volumes was calculated and used as an index for determination of SVL size at sexual maturity and possible variation of testicular size with season.
2. Dimensions of ovarian follicles (F). In most specimens the diameters of only the largest three follicles in each ovary on left (L) or right (R) side and/or eggs in oviducts were measured. In the pilot study of *N. asper,* only the largest follicle on each side was measured, but in a representative sample of specimens all the follicles visible at x40 magnification were measured. Follicles were numbered in decreasing order of diameters (FL1, FL2, FL3... on left and FR1, FR2, FR3... on right). The volume of follicles was calculated using the formula for determination of the volume of a sphere ($V = 4*\pi*r^3/3$, where V = volume in mm^3, and r = follicular radius in mm), (the volumes of eggs are treated in Chapter 5). The measurements of FL1 and FR1 were summed and used as an index for comparison with the SVL.

Table A4.1 *Nephrurus amyae* measurements of gonads

	TESTES				OVARIES			
Gonad Character	Mean	Range	STD	N	Mean	Range	STD	N
Lt Testis Length	4.97	1.77-6.32	1.87	5				
Lt T Width	3.79	1.10-6.00	1.76	5				
Lt T Depth	2.37	0.77-4.50	1.42	5				
Lt T Volume	33.2	0.79-89.1	33.5	5				
Rt T Length	4.85	1.85-6.1	1.81	5				
Rt T Width	3.91	1.07-5.8	1.82	5				
Rt T Depth	1.99	0.67-3.60	1.14	5				
Rt T Vol.	26.9	0.69-66.7	24.7	5				
TestesVol. Lt+Rt	60.0	1.48-156	58.2	5x2				
^{3}/ TVol	3.46	1.14-5.38	1.54	5x2				
^{3}/TVol as % SVL	3.80	1.62-5.22	1.36	5x2				
FL1 Diameter					5.61	1.95-13.4	3.99	6
FL1 Volume					246	3.88-1260	498	6
FL 2D					2.85	0.45-3.74	1.22	6
FL 3D					2.16	0.21-2.72	0.977	6
FR1 Diameter					4.69	1.15-14.0	4.31	7
FR1 Volume					226	.796-1437	534	7
FR 2D					2.63	.32-3.55	1.19	6
FR 3D					2.52	2.24-2.81	0.248	5
FL1+FR1 as % SVL					9.95	5.65-23.48	6.80	6x2

Table A4.2 *Nephrurus asper* measurements of gonads

	TESTES				OVARIES			
Gonad Character	Mean	Range	STD	N	Mean	Range	STD	N
Lt Testis Length	5.13	1.20-7.50	1.63	21				
L T Width	3.95	0.90-6.00	1.43	21				
L T Depth	2.89	.370-4.70	1.04	21				
L T Volume	45.5	.096-108	26.8	21				
Rt Testis Length	5.79	1.10-11.3	2.26	21				
R T Width	4.06	.420-6.10	1.33	21				
R T Depth	2.83	0.35-4.30	1.01	21				
R T Volume	43.4	.085-122	23.0	21				
TestesVol Lt+Rt	91.8	.180-231	61.8	21x2				
$^{3}\sqrt{}$ TVol Lt+Rt	4.20	.560-6.13	1.32	21x2				
$^{3}\sqrt{}$ TVol as % SVL	5.10	1.59-6.89	1.20	21x2				
FL1 Diameter					9.44	0.50-28.9	11.4	11
FL1 Volume					229	.064-1468	515	11
FR1 Diameter					11.5	0.60-27.5	11.9	10
FR1 Volume					336	.113-1564	628	10
FL1+FR1as %SVL					11.2	1.15-26.7	11.1	10x2

Table A4.3 *Nephrurus deleani* measurements of gonads

	TESTES				OVARIES			
Gonad Character	Mean	Range	STD	N	Mean	Range	STD	N
Lt Testis Length	3.71	1.80-5.50	1.40	5				
L T Width	1.94	.880-3.20	.830	5				
L T Depth	1.28	.040-2.70	.983	5				
L T Volume	7.78	.033-24.9	9.91	5				
Rt Testis Length	3.89	1.66-5.70	1.50	5				
R T Width	2.40	.920-4.00	1.17	5				
R T Depth	1.12	.060-1.80	.661	5				
R T Volume	8.02	.048-21.5	8.13	5				
TestesVol. Lt+Rt	15.8	.081-46.4	18.0	5x2				
^{3}/TVol .Lt+Rt	2.12	.433-3.59	1.14	5x2				
^{3}/TVol as % SVL	3.53	1.11-5.36	1.53	5x2				
FL1 Diameter					4.02	2.40-7.00	2.58	3
FL1 Volume					65.6	7.24-180	98.8	3
FL2 D					2.30	2.30		2
FL3 D					1.85	1.85		1
FR1 Diameter					3.23	2.98-3.7	0.41	3
FR1 Volume					18.2	13.6-26.5	7.23	3
FR2 D					2.36	1.41-3.23	0.92	3
FR3 D					1.9	1.9		1
FL1+FR1 as %SVL					9.86	6.80-15.2	4.6	3x2

Table A4.4 *Nephrurus laevissimus* measurements of gonads

	TESTES				OVARIES			
Gonad Character	Mean	Range	STD	N	Mean	Range	STD	N
Lt Testis Length	4.37	0.5-7.2	1.65	124				
L T Width	2.85	0.3-5.40	1.14	123				
L T Depth	1.97	0.15-3.6	0.86	123				
L T Volume	17.8	0.02-62.3	12.5	123				
Rt Testis Length	4.29	0.50-6.90	1.60	123				
R T Width	3.01	0.29-1.24	1.24	123				
R T Depth	1.96	0.15-3.90	0.89	123				
R T Volume	18.6	0.02-70.8	14.0	123				
TestesVol Lt+Rt	36.3	0.03-111	25.2	123x2				
^{3}/TVol Lt+Rt	3.01	.314-6.18	1.18	123x2				
^{3}/TV as % SVL	4.80	.766-14.7	1.65	123x2				
FL1 Diameter					4.83	0.30-22.0	5.26	90
FL1 Volume					188	.014-1150	223	82
FL2 D					1.07	0.20-4.20	0.91	79
FL3 D					1.57	0.2-3.50	0.66	66
FR1 Diameter					4.7	0.27-23.8	5.14	89
FR1 Volume					92.0	0.010-951	217	83
FR2 D					2.0	0.2-4.70	0.99	84
FR3 D					1.52	0.20-2.70	0.62	71
FL1+FR1 as %SVL					12.5	1.60-54.2	12.3	90x2

Table A4.5 *Nephrurus levis* measurements of gonads

	TESTES				OVARIES			
Gonad Character	Mean	Range	STD	N	Mean	Range	STD	N
Lt Testis Length	4.92	0.6-7.3	1.35	76				
L T Width	3.27	5.0-0.83	0.94	75				
L T Depth	2.29	0.4-3.9	0.81	75				
L T Volume	23.2	.302-54.2	13.9	75				
Rt Testis Length	4.88	0.71-7.5	1.37	73				
R T Width	3.49	0.7-5.9	1.14	73				
R T Depth	2.26	0.4-4.0	0.81	73				
R T Volume	24.7	0.26-74.9	16.8	73				
TestesVol. Lt+Rt	47.7	0.57-126	29.7	73x2				
^{3}/TVol Lt+Rt	3.34	0.83-5.01	1.05	73x2				
^{3}/TVol as % SVL	5.12	2.24-7.63	1.12	73x2				
FL1 Diameter					5.86	0.12-26.2	6.18	54
FL1 Volume					665	.0009-9417	1864	54
FL2 D					2.94	1.00-8.57	2.57	7
FL3 D					2.02	1.45-4.67	1.41	5
FR1 Diameter					6.78	0.11-27.7	7.66	54
FR1 Volume					1129	.0007-1129	2805	53
FR2 D					2.65	0.85-5.32	1.65	7
FR3 D					1.82	0.50-3.39	1.02	6
LF1+FR1 as %SVL					15.4	0.5-54.1	14.1	53x2

Table A4.6 *Nephrurus sheai* measurements of gonads

	TESTES				OVARIES			
Gonad Character	Mean	Range	STD	N	Mean	Range	STD	N
Lt Testis Length	4.36	2.7-6.30	1.36	11				
L T Width	3.18	1.51-4.6	1.20	11				
L T Depth	2.19	0.93-3.5	0.92	11				
L T Volume	21.3	1.99-51.1	19.2	11				
Rt Testis Length	4.34	2.35-6.5	1.58	11				
R T Width	3.37	2.11-4.50	0.93	11				
R T Depth	2.14	0.76-3.50	0.91	11				
R T Volume	21.7	2.04-51.1	19	11				
TestesVol Lt+Rt	43.0	4.03-102	38	11x2				
^{3}/TVol Lt+Rt	3.20	1.59-4.68	1.08	11x2				
^{3}/TVol as % SVL	4.18	2.97-5.70	0.83	11x2				
FL1 Diameter					9.58	0.53-28.1	10.1	14
FL1 Volume					166	.078-796	277	11
FL2 D					2.65	0.84-3.90	1.00	6
FL3 D					1.50	0.23-2.05	0.85	4
FR1 Diameter					9.61	0.53-30.9	10.7	14
FR1 Volume					146	.078-860	269	11
FR2 D					1.94	0.41-3.20	1.14	6
FR3 D					1.22	0.40-2.07	0.94	4
FL1+FR1 as %SVL					17.8	1.80-53.0	18.4	14x2

Table A4.7 *Nephrurus stellatus* measurements of gonads

	TESTES				OVARIES			
Gonad Character	Mean	Range	STD	N	Mean	Range	STD	N
Lt Testis Length	4.68	2.49-6.44	1.76	6				
L T Width	2.70	0.97-4.00	1.11	6				
L T Depth	1.85	0.64-2.90	1.09	6				
L T Volume	18.1	.809-38.3	16.9	6				
Rt Testis Length	4.69	2.65-6.51	1.79	6				
R T Width	2.84	1.40-4.10	1.16	6				
R T Depth	1.69	0.32-2.80	1.02	6				
R T Volume	17.8	.673-37.9	16.7	6				
TestesVol Lt+Rt	35.9	1.48-76.1	33.6	6x2				
^{3}/TVol Lt+Rt	2.87	1.14-4.24	1.33	6x2				
^{3}/TVol as % SVL	4.42	2.10-6.08	1.68	6x2				
FL1 Diameter					8.35	8.00-8.70		2
FL1 Volume					306	268-344		2
FR1 Diameter					9.00	8.80-9.20		2
FR1 Volume					382	357-408		2
FL1+FR1as % SVL					20.5	20.0-21.1		2x2

Table A4.8 *Nephrurus vertebralis* measurements of gonads

	TESTES				OVARIES			
Gonad Character	Mean	Range	STD	N	Mean	Range	STD	N
Lt Testis Length	4.52	1.66-6.60	1.42	33				
L T Width	2.73	0.72-4.55	1.02	33				
L T Depth	1.77	0.38-3.11	.769	33				
L T Volume	14.5	.318-34.8	11.3	33				
Rt Testis Length	4.53	1.36-6.78	1.38	33				
R T Width	2.84	0.91-4.50	1.00	33				
R T Depth	1.72	0.31-3.10	.669	33				
R T Volume	14.7	.261-34.7	10.6	33				
Testes Vol. Lt+Rt	29.2	0.58-68.8	21.8	33x2				
^{3}/TVol Lt+Rt	2.81	0.83-4.10	.948	33x2				
^{3}/TVol as % SVL	4.53	2.07-5.91	.9960	33x2				
FL1 Diameter					4.16	0.20-13.2	4.25	14
FL1 Volume					180	.004-1196	363	14
FL2 D					2.38	0.38-5.90	1.67	10
FL3 D					1.93	0.48-4.80	1.32	9
FR1 Diameter					4.02	0.020-13.6	3.92	14
FR1 Volume					162	.004-1317	368	14
FR2 D					2.18	0.39-4.9	1.32	11
FR3 D					1.63	0.30-2.72	.778	10
FL1+FR1 as %SVL					4.77	0.98-12.0	3.28	12x2

Table A4.9 *Nephrurus wheeleri* measurements of gonads

	TESTES				OVARIES			
Gonad Character	Mean	Range	STD	N	Mean	Range	STD	N
Left Testis L	5.77	1.04-7.58	1.82	16				
L T Width	3.11	0.66-4.83	1.14	16				
L T Depth	1.81	0.43-2.94	.713	16				
L T Volume	20.6	.155-50.5	15.5	16				
Rt Testis Length	6.02	1.05-7.92	1.92	16				
R T Width	3.44	0.47-5.26	1.27	16				
R T Depth	1.96	0.35-3.02	.793	16				
R T Volume	25.3	0.09-52.6	15.6	16				
TestesVol Lt+Rt	45.9	.245-101	28.5	16x2				
^{3}/TVol Lt+Rt	3.35	.626-4.66	1.01	16x2				
^{3}/TVol as % SVL	4.47	1.53-5.91	1.08	16x2				
FL1 Diameter					5.30	1.29-18.2	5.43	16
FL1 Volume					386	1.12-3157	968	16
FL2 D					2.49	0.93-3.82	1.01	14
FL3 D					1.74	0.57-2.64	.728	14
FR1 Diameter					5.29	1.24-18.8	5.72	17
FR1 Volume					407	1.00-3479	1062	16
FR2 D					2.35	.998-3.70	.921	14
FR3 D					1.80	0.41-2.68	.793	14
FL1+FR1 as %SVL					12.2	4.22-40.1	12.0	14x2

Table A4.10 *Underwoodisaurus milii measurements of gonads*

	TESTES				OVARIES			
Gonad Character	Mean	Range	STD	N	Mean	Range	STD	N
Lt Testis Length	4.36	0.80-9.40	2.48	108				
L T Width	2.65	0.30-7.10	1.82	107				
L T Depth	1.86	0.20-4.70	1.52	107				
L T Volume	25.4	.031-132	31.9	107				
Rt Testis Length	4.45	1.0-9.10	2.51	109				
R T Width	2.79	0.30-6.60	1.89	108				
R T Depth	1.77	0.20-4.70	1.89	108				
R T Volume	25.3	.031-133	30.8	108				
TestesVol Lt+Rt	53.6	.063-264	62.7	97x2				
^{3}/TVol Lt+Rt	2.97	.397-6.42	1.81	97x2				
^{3}/TVol as % SVL	4.09	0.886-9.03	1.95	97x2				
FL1 Diameter					4.37	.250-23.4	5.29	95
FL1 Volume					69.8	.008-1406	189	89
FL2 D					2.14	.460-4.26	1.09	17
FL3 D					1.68	0.40-3.10	0.70	14
FR1 Diameter					4.29	.150-23.5	4.93	95
FR1 Volume					74.2	.002-1376	192	89
FR2 D					2.08	.290-3.50	.974	15
FR3 D					1.54	.170-2.51	.637	11
FL1+FR1 as %SVL					12.0	.952-65.3	13.5	46x2

Table A4.11 *Underwoodisaurus sphyrurus* measurements of gonads

	TESTES				OVARIES			
Gonad Character	Mean	Range	STD	N	Mean	Range	STD	N
Lt Testis Length	5.78	1.22-10.0	2.82	12				
L T Width	2.75	0.57-4.50	1.30	12				
L T Depth	1.96	0.33-3.15	1.02	12				
L T Volume	37.0	0.23-74.0	30.0	12				
Rt Testis Length	4.88	1.25-10.3	2.43	12				
R T Width	2.99	0.30-5.50	1.57	12				
R T Depth	1.74	0.16-3.15	0.90	12				
R T Volume	18.4	.031-42.4	16.0	12				
TestesVol Lt+Rt	44.0	.203-89.9	35.1	12x2				
^{3}/TVol Lt+Rt	3.07	.588-4.48	1.42	12x2				
^{3}/TVol as % SVL	4.78	1.55-7.15	1.94	12x2				
FL1 Diameter					4.63	0.5-11.4	3.73	13
FL1 Volume					158	.066-777	241	13
FL2 D					3.13	2.61-3.64		2
FL3 D					2.21	2.03-2.38		2
FR1 Diameter					4.51	0.50-11.9	3.95	13
FR1 Volume					171	.065-882	281	13
FR2 D					3.50	3.18-3.82		2
FR3 D					2.86	2.80-2.91		2
FL1+FR1 as %SVL					12.6	1.49-23.8	10.1	13x2

Table A4.12 Minimum snout-vent length of mature *Nephrurus* and *Underwoodisaurus* specimens and percentage of maximum size for males and females.

	MALES				FEMALES			
SPECIES	SVL mm	N_1*	%MAX. SVL	N_2*	SVL mm	N_1*	%MAX. SVL	N_2*
N. amyae	69	6	63	10	97	8	71#	10
N. asper	63	22	61	29	76	15	65	27
N. deleani	42	5	62#	8	63	3	71#	12
N. laevissimus	49	122	61	131	66	109	69	124
N. levis	50	76	62	94	74	54	75	79
N. sheai	63	11	63	11	86	14	71#	21
N. stellatus	48	6	62#	19	61	2	71#	15
N. vertebralis	45	33	58	38	64	14	71#	16
N. wheeleri	51	12	62#	16	78	14	76	17
MEANS			**61.3**				**71.3**	
*U. milii***	57	67	60	109	72	63	65	95
U. sphyrurus	44	12	60	13	55	13	67	12
MEANS			**60.0**				**66.0**	

* N_1 and N_2 are the number of specimens examined in determination of the minimum size at sexual maturity and, the number of specimens examined in determination of maximum size respectively in males and females, (see Chapter 4 for methods).

** One exceptionally precocious female *U. milii* was found with enlarged ovarian follicles at 61 mm SVL, or 55% of maximum (not used in calculations).

These values are probably imprecise because the range of intermediate sized specimens in these groups was inadequate (not used in calculations).

Appendix 5

5.1 Preferred Habitats of *Nephrurus* and *Underwoodisaurus* Geckos

N. amyae

The preferred habitat for *N. amyae* is described as open woodland with a rocky substrate and sometimes associated with a scattered *Triodia* understorey (Couper and Gregson, 1994).

N. asper

The habitat for *N. asper* has been variously described as desert, hummock grassland, low open woodland, low woodland, woodland, open forest or tall shrub-land (Annable, 1992; Couper and Gregson, 1994; Cogger, 1996). This species is often associated with rocky areas (Cogger, 2000; Ehmann, 1992). The distribution of this species covers much of the warm arid to semi-arid regions of central and northern Queensland but also extends into the monsoonal Cape York Peninsular (Couper and Gregson, 1994; personal observation).

N. deleani

The habitat for *N. deleani* is described as being 'desert' (Harvey, 1983) and as Mulga dominated sand dunes with low *Sclerolaena* saltbush (Ehmann, 1992).

N. laevissimus

The habitat for *N. laevissimus* is described as being desert, sand dune ridges, low open shrubland and tall shrubland, often associated with *Triodia* (Storr, 1963; Delean and Harvey, 1981; Pianka, 1986; Ehmann, 1992; Cogger, 1996).

N. levis

The preferred habitat for *N. levis* is variously described as being desert, hummock

grassland, tussock grassland, low open shrubland, low shrubland and tall shrubland (Storr, 1963; Warburg, 1966, Drury, 1996). This species is often associated with *Triodia* (Ehmann, 1992; Cogger, 1996: personal observation).

N. sheai

The habitat for *N. sheai* is described as being rocky areas of open woodland scattered *Triodia* or grass understorey (Couper and Gregson, 1994).

N. stellatus

The preferred habitat for *N. stellatus* consists of low shrubland, open heath, open scrub and tall shrubland (Storr, 1968). It is sometimes found in association with *Triodia* and mallee eucalypts (Ehmann, 1992) and also in arid rocky areas (Mark Hutchinson personal communication; personal observation).

N. vertebralis

The habitat for *N. vertebralis* is described as being desert, hummock grassland, and low open shrubland (Storr, 1963) or arid shrubland (Cogger, 1996). This species may also be found in association with *Triodia*, chenopod or Mulga shrubland (Ehmann, 1992).

N. wheeleri

The preferred habitat for *N. wheeleri* is described as hummock grassland, low open shrubland and tall shrubland. *N. wheeleri* is often found on rocky or stony ground in association with *Triodia* (Ehmann, 1992; personal observation).

U. milii

U. milii is commonly found in rocky areas but may also be found in a range of habitats including wet coastal heathlands, open wet sclerophyll forests, grasslands and arid scrubs (Wilson and Knowles, 1988; Ehmann, 1992; Cogger, 1996; Spark, 1997; personal observation)

U. sphyrurus

U. sphyrurus is restricted to cool, drier highlands with exfoliating rocky outcrops in *Eucalyptus* woodlands with deep leaf litter (Wilson and Knowles, 1988; Ehmann, 1992; Cogger, 1996; Spark, 1997; personal observation).

APPENDIX 6

NEPHRURUS AND *UNDERWOODISAURUS* EGG DATA TABLES,
(including some diplodactyline and gekkonine comparative data)

See Chapters 3, 4 and 5 for methodologies, analyses and discussion relating to egg data. There are no data yet available for *N. vertebralis* or *N. wheeleri* eggs. Volumes and densities (for *N. levis* and *U. milii*) have been calculated with the assumption of a prolate ellipsoidal egg shape.

Table A6.1 ***N. Amyae*** **eggs***

CHARACTER	MEAN	RANGE	STD	N
Initial Length (mm)	31.7	28-37	3.55	6
Initial Breadth (mm)	16.7	16.1-17.4	0.524	6
Length/Breadth Ratio	1.89	1.68-2.30	0.242	6
Length +Breadth as % SVL	35.3	32.3-38.8	2.51	6
Volume (mL)	4.62	3.90-5.02	0.497	6
$3\sqrt{}$Volume	16.0	15.7-16.1	0.141	6
Initial Wet Mass (g)	5.23	4.66-5.80	0.555	4
Maximum Mass (g)	5.63	5.12-6.14	0.721	2
Mass Increase %*	18.0	9.81-26.3	11.6	2
Initial Surface Area (cm^2)	16.6	14.3-18.7	1.74	6
Maximum Area (cm^2)	17.3	17.017.5	0.352	2
Area Increase %*	2.09	1.73-2.44	0.501	2
Maximum Length (mm)	31.3	30.8-31.7	0.636	2
Length Increase %*	0.639	0.000-1.28	0.904	2
Maximum Breadth (mm)	17.6	17.6-17.6	0.000	2
Breadth Increase %*	1.44	1.15-1.73	0.413	2
Relative Clutch Mass %	26.0	24.8-27.0	1.12	4
Incubation at 30°C (days)	56			1
Hatchling SVL (mm)	52.6			1
Hatchling Tail (mm)	11.8			1
Hatchling Mass (g)	4.12			1
Hatchling Mass as % Egg Mass	88.5			1

* Data and derivatives based on measurements supplied by Mr Robert Porter for four of the six eggs.

Table A6.2 *N. asper* eggs

CHARACTER	MEAN	RANGE	STD	N
Initial Length (mm)	26.5	19.3-29.7	2.49	18
Initial Breadth (mm)	15.3	12.9-16.7	0.985	18
Length/Breadth Ratio	1.73	1.50-1.83	0.074	18
Length +Breadth as % SVL	35.5	27.3-39.3	2.92	18
Volume (mL)	3.31	1.68-4.34	0.663	18
$3\sqrt{}$Volume	1.48	1.19-1.63	0.109	18
Initial Wet Mass (g)	3.69	2.56-4.78	0.593	18
Maximum Mass (g)	4.33	3.90-4.78	0.325	9
Mass Increase %	23.0	4.23-40.2	10.9	8
Initial Area (cm^2)	13.5	11.2-15.6	1.19	18
Maximum Area (cm^2)	14.2	13.2-15.6	0.974	7
Area Increase %	7.91	-0.11-19.7	8.51	7
Maximum L (mm)	27.0	24.9-29.7	1.57	7
Length Increase %	0.189	-1.10-0.81	0.756	7
Maximum Breadth (mm)	16.8	16.1-18.4	0.763	7
Breadth Increase %	8.08	0.00-18.8	8.03	7
Relative Clutch Mass %	28.1	24.6-30.3	2.61	4

TABLE A6.3 *Laevissimus* eggs*

CHARACTER	MEAN	RANGE	STD	N
Initial Length (mm)	19.5	18-22	1.309	8
Initial Breadth (mm)	10.1	9-11	0.835	8
Length/Breadth Ratio	1.93	1.73-2.20	0.168	8
Length +Breadth as % SVL	31.2	28.4-33.7	1.86	8
Volume (mL)	1.06	0.763-1.27	0.200	8
$3\sqrt{}$Volume	1.02	0.914-1.08	0.066	8
Initial Wet Mass (g)	1.23	0.886-1.47	0.233	8
Initial Area (cm^2)	6.21	5.09-6.91	0.744	8

- All data and derivatives based on measurements supplied by Mr Neil Sonnemann.

Table A6.4 *N. deleani* eggs

CHARACTER	MEAN	RANGE	STD	N
Laying Dates		9/11-21/12		
Initial Length (mm)	23.2	20.7-25.4	1.58	8
Initial Breadth (mm)	12.7	11.4-13.4	0.624	8
Length/Breadth Ratio	1.83	1.66-2.04	0.129	8
Length +Breadth as % SVL	40.3	37.3-42.9	2.13	8
Volume (mL)	1.96	1.59-2.32	0.270	8
Bicone Ratio	0.299	0.036-0.488	0.235	3
$3\sqrt{}$Volume	1.25	1.17-1.32	0.058	8
Initial Wet Mass (g)	2.10	1.71-2.52	0.277	8
Maximum Mass (g)	2.46	2.35-2.69	0.107	8
Mass Increase %	19.4	-2.19-47.3	18.7	8
Initial Surface Area (cm^2)	9.26	8.13-10.4	0.903	8
Maximum Area (cm^2)	10.0	8.35-11.1	0.844	8
Increase in Area %	9.01	0.00-34.7	13.4	8
Maximum Length (mm)	23.6	22.4-25.6	1.09	8
Increase in Length %	1.85	-2.14-8.49	4.17	8
Maximum Breadth (mm)	13.5	11.4-15.4	1.15	4
Increase in Breadth %	6.76	-0.15-24.2	8.86	8
Relative Clutch Mass	36.2	33.5-38.4	2.04	4
Sinus Terminalis (mm)$^{\varphi}$	12	10-15	2.37	6
Incubation at 25°C (days)	85	85	NA	1
Hatchling SVL (mm)	33.2	33.2	NA	1
Hatchling Tail (mm)	13.4	13.4	NA	1
Hatchling Mass (g)	1.34	1.34	NA	1
Hatchling Mass as % Egg Mass	83	83	NA	1
Hatchling Water content (g)	1.73	1.73	NA	1
Hatchling % Water	82.4	82.4	NA	1
Egg % Water	79.5	71.4-90.2	10.6	3
Egg Dry Mass (g)	0.616	0.549-0.682	NA	2
Egg Dry Mass % Initial Mass	25.9	23.1-28.6	NA	2
Dry Shell (mg)	48.7	31.9-65.5	NA	2
Dry Mass (Excluding Shell) (g)	0.584	0.517-0.650	NA	2
Total Lipids (mg/Egg)	120	111-129	7.35	3
Lipid % Dry Mass	31.1	30.9-31.3	0.307	3
Lipid (kJ/Egg)	7.50	6.74-8.26	NA	2
Protein % Dry Egg	52.7	48.5-55.1	NA	2
Energy kJg^{-1} of Dry Mass	16.2	15.74-16.7	NA	2
Total kJ/g	20.4	12.34-24.9	6.98	3
kJ/Egg	9.89	1.11-17.4	.634	3
kJ/clutch	13.9	6.71-21.1	NA	2
Ash % of Dry Mass	3.18	0.76-5.69	2.47	3

$^{\varphi}$ Maximum straight-line diameter (perpendicular to long axis of egg).

TABLE A6.5 *N. levis* eggs

CHARACTER	MEAN	RANGE	STD	N
Initial Length (mm)	24.5	20.8-27.4	1.44	41
Initial Breadth (mm)	13.8	11.5-16.1	1.02	41
Length/Breadth Ratio	1.78	1.54-2.00	0.100	41
Length +Breadth as % SVL	39.1	33.8-43.4	2.28	41
Volume (mL)	2.46	1.50-3.37	0.464	41
$3\sqrt{}$Volume	1.35	1.15-1.50	0.085	41
Initial Wet Mass (g)	2.48	1.66-3.31	0.399	46
Calculated Density	1.03	0.858-1.127	0.065	41
Measured Density	1.168	1.162-1.175	NA	2
Bicone Ratio	0.245	0.135-0.479	0.123	7
Maximum Mass (g)	2.98	0.689-4.71	0.893	41
Increase in Mass %	18.2	-72.5-103	33.2	24
Increase in Mass % hatched	30.9	-5.4-103	29.4	13
Initial Area (cm^2)	10.7	7.84-13	1.29	37
Maximum Area (cm^2)	11.4	8.40-16.3	1.92	18
Increase in Surface Area %	12.6	-6.75-59.2	15.9	18
Maximum L (mm)	24.4	20.4-30.2	2.14	18
Increase in Length %	2.19	-2.01-21.7	5.68	18
Maximum W (mm)	14.8	12.9-17.6	1.42	18
Increase in Breadth %	5.90	0.000-17.8	5.36	18
Relative Clutch Mass	29.5	24.8-37.2	4.51	7
Incubation 25°C&-100kPa(d)	94.8	69-111	17.6	5
Incubation 25°C&-450kPa(d)	97.7	86-112	13.2	3
Incubation 30°C&-100kPa(d)	48.7	19-64	25.7	3
Incubation 30°C&-450kPa(d)	50.0	22-62	14.8	6
Hatchling SVL (mm)	36.91	30.0-42.1	2.67	20
Hatchling Tail (mm)	15.8	11.7-18.1	2.14	19
Hatchling Mass (g)	1.85	0.876-2.40	0.413	18
Hatchling Mass as % Initial Mass	82.5	69.0-91.0	7.28	15
Egg % Water	79.6	65.6-92.1	7.89	11
Egg Dry Mass (g)	0.414	0.194-0.802	0.204	11
Dry Mass Excluding Shell (g)	0.323	0.155-0.489	0.125	11
Lipid % Dry Mass	31.9	19.5-42.2	6.73	13
Lipid kJ/Egg	5.42	2.22-10.5	3.06	11
Protein % Dry Mass	56.0	47.6-63.8	6.92	9
Total kJ/g	16.3	8.63-26.4	5.01	11
kJ/Egg	11.4	5.2-15.0	3.18	7
Ash % of Dry Mass	6.64	4.90-8.38	1.28	7

TABLE A6.6 *U. milii* eggs

CHARACTER	MEAN	RANGE	STD	N
Initial Length (mm)	23.2	20.1-28.2	1.71	83
Initial Breadth (mm)	12.2	8.75-15.1	0.923	83
Length/Breadth Ratio	1.91	1.65-2.33	0.118	83
Length +Breadth as % SVL	34.8	28.5-40.7	2.37	83
Volume (mL)	1.84	0.816-2.97	0.377	83
$3\sqrt{}$Volume	1.22	0.934-1.44	0.084	83
Initial Wet Mass (g)	1.91	0.775-3.02	0.396	83
Density (g/mL)	1.08	0.875-1.62	0.099	73
Bicone Ratio	0.359	0.130-0.784	0.191	12
Maximum Mass (g)	2.65	1.44-3.94	0.598	53
Mass Increase %	34.4	-4.67-137	27.2	52
Mass Increase % (hatched eggs)	39.2	12.9-95.9	20.3	27
Initial Area (cm^2)	8.96	5.59-11.8	1.21	78
Maximum Area (cm^2)	10.3	7.62-12.0	1.09	37
Area Increase %	15.3	2.01-43.6	8.87	37
Maximum Length (mm)	23.4	20.2-26.0	1.31	40
Length Increase %	0.631	-6.10-7.04	2.71	40
Maximum Breadth (mm)	14.0	11.9-15.6	1.04	37
Breadth Increase %	14.7	1.61-34.2	7.34	37
Relative Clutch Mass	24.7	14.7-34.9	6.04	15
Incubation 25°C&-100kPa(d)	83.3	79-87	4.35	4
Incubation 25°C&-450kPa(d)	74.4	62-83	7.44	8
Incubation 30°C&-100kPa(d)	53	50-56	3.00	3
Incubation 30°C&-450kPa(d)	51.5	50-53	2.12	2
Hatchling SVL (mm)	38.0	31.7-43.5	3.07	31
Hatchling Tail (mm)	25.1	20.0-30.0	2.64	31
Hatchling Mass (g)	1.67	0.973-2.05	0.294	33
Hatchling Mass as % Initial Mass	83.1	54.0-106	11.3	31
Egg % Water	81.1	73.0-96.2	5.36	38
Dry Mass (g)	0.318	0.060-0.574	0.107	39
Dry Mass Excluding Shell (g)	0.336	0.102-0.574	0.103	36
Lipid % Dry Mass	35.7	28.6-52.7	5.72	36
Lipid kJ/Egg	4.67	2.06-7.59	1.47	34
Protein % Dry Mass	50.9	41.6-58.0	4.12	27
Total kJ/g	25.2	19.0-27.9	2.00	22
Total kJ/Egg	7.95	1.43-13.0	3.00	26
Ash %	6.03	2.66-14.78	2.38	31
Calculated Density	1.08	0.875-1.62	0.099	73

TABLE A6.7 *N. stellatus* eggs*

CHARACTER	MEAN	RANGE	STD	N
Initial Length (mm)	22.4	21.8-23.6	0.656	6
Initial Breadth (mm)	12.5	11.5-13.7	0.866	6
Length/Breadth Ratio	1.79	1.72-1.90	0.083	6
Length +Breadth as % SVL	40.6	38.7-43.4	1.72	6
Volume (mL)	1.85	1.51-2.32	0.307	6
$3\sqrt{}$Volume	1.23	1.15-1.32	0.067	6
Initial Wet Mass (g)	1.99	1.96-2.02	0.042	6
Initial Area (cm^2)	8.82	7.88-10.16	0.854	6

- All data and derivatives based on measurements supplied by Mr Robert Porter and Mr Peter Page.

TABLE A6.8 *U. sphyrurus* eggs

CHARACTER	MEAN	RANGE	STD	N
Initial Length (mm)	21.7	21.3-22.1	NA	2
Initial Breadth (mm)	10.9	10.7-11.1	NA	2
Length/Breadth Ratio	1.99	1.92-2.07	NA	2
Length +Breadth as % SVL	32.0	31.8-32.2	NA	2
Volume (mL)	1.35	1.32-1.37	NA	2
$3\sqrt{}$Volume	1.11	1.10-1.11	NA	2
Initial Wet Mass (g)	1.53	1.51-1.55	NA	2
Initial Area (cm^2)	7.43	7.43-7.43	NA	2
Bicone Ratio	0.133	0.038-0.228	NA	2
Relative Clutch Mass	30.8	30.8	NA	1

Table A6.9 Mean Relative Clutch Masses of Diplodactyline and Gekkonine Species (all 2-egg clutches)
The Relative Clutch Mass is measured as the total egg wet mass/post-oviposition maternal wet mass expressed as a percentage (values in the literature using preserved diplodactyline material (e.g. Pianka and Pianka, 1976) excluded), (N = number of clutches).

DIPLODACTYLINE SPECIES	**RCM**	**ARCSINE**	**N**	**REFERENCE**
Carphodactylus laevis	16.0	0.161	4	R. Porter (personal communication).
Diplodactylus taenicauda	21.1	0.213	33	R. Porter (p. c.)
Nephrurus amyae	26.0	0.263	4	T. Annable (this study); R. Porter (p. c.)
Nephrurus asper	28.1	0.285	4	T. Annable (this study)
Nephrurus deleani	36.2	0.370	4	T. Annable (this study)
Nephrurus levis	29.5	0.299	7	T. Annable (this study)
Oedura castelnaui	19.9	0.200	10	Bustard, 1967c; R. Porter (p. c.)
Oedura coggeri	24.6	0.249	5	Robert Porter (personal communication).
Oedura lesueurii	31	0.315	1	Bustard, 1967c
Oedura marmorata	27	0.273	3	Bustard, 1967c
Oedura monilis	35.2	0.360	6	Bustard, 1967c
Oedura tryoni	26	0.263	1	Bustard, 1967c
Phyllurus championae	30.6	0.311	1	Couper et al., 2000
Phyllurus platurus	25.3	0.256	2	Greer, 1989; T. Annable (this study); R. Porter (p. c.)
Strophurus elderi	35.5	0.363	2	Bustard, 1965a; R. Porter (p. c.)
Underwoodisaurus milii	24.7	0.250	15	T. Annable (this study)
Underwoodisaurus sphyrurus	30.8	0.313	1	T. Annable (this study)
MEAN RCM	**27.5**	**0.279**	**18**	
GEKKONINE SPECIES				
Chondrodactylus angulifer	12.2	0.122	2	Pianka and Huey, 1978
Christinus marmoratus #	33	0.336	2	Bustard, 1965a; R. Porter (p. c.)
Colopus wahlbergii	8.2	0.082	18	Pianka and Huey, 1978
Cosymbotus platyurus	8	0.080	?	Shine, 1992
Cyrtodactylus louisiadensis	162	0.162	2	T. Annable (this study)
Gehyra dubia	25	0.253	2	Bustard, 1965a; R. Porter (p.c.)
Gekko gecko	10.6	0.106	?	Packard & Hirsch, 1989
*Hemidactylus fasciatus***	13	0.130	?	Shine, 1992
*Hemidactylus frenatus***	6	0.060	?	Shine, 1992
*Hemidactylus mabouia***	16.1	0.162	1	Vitt, 1986
Heteronotia binoei	30	0.305	5	Bustard, 1968; Pianka, 1986; T. Annable (this study)

Lepidodactylus lugubris	11.6	0.116	?	Schwaner, 1980
Lygodactylus klugei	20.6	0.106	47	Vitt, 1986
Pachydactylus bibroni	10.6	0.111	16	Pianka and Huey, 1978
Pachydactylus capensis	11.1	0.131	15	Pianka and Huey, 1978
Phyllopezus pollicaris	13.1	0.152	7	Vitt, 1986
Ptenopus garrulus	8.8	0.088	?	Pianka and Huey, 1978
MEAN RCM	**14.6**	**14.7**	**19**	

Christinus marmoratus frequently lays only one egg per clutch but is predominantly a two-eggs per clutch species (Doughty and Thompson, 1998). p. c. = personal communication.

APPENDIX 7

Gekkonine, Sphaerodactyline, Diplodactyline and Eublepharine Gecko SVLs & Egg Sizes

The egg dimensions (in mm) are the averages of maximum and minimum values (including subspecies values).
In the case of many gekkonine eggs no account has been taken of the flat side(s) of the egg where attached to second egg and/or substrate.
No data are available for the smallest carphodactyline gecko *Bavayia validiclavis* or diplodactyline species *Diplodactylus occultus*.
SVL measurements are the maximum found for females or alternatively for the species.

Table A7.1

GEKKONINE GECKOS	SVL	L	W	L+W	L/W	L+W* 100/SVL	Arcsine	Source
Afroedura pondolia	47	9.9	8.2	18.1	1.2	38.5	0.395	Hewitt, 1925; Douglas, 1990
Ailuronyx seychellensis	107	19	16	34.5	1.2	32.2	0.328	McKeown and Miller, 1985; Henkel, F. W. and Zobel, 1987
Alsophylax laevis	45	9.4	6.6	16	1.4	35.6	0.364	Seufer, 1991; Szczerbak and Golubev, 1996
Alsophylax loricatus	32.8	8.7	5.8	14.5	1.5	44.2	0.458	Szczerbak and Golubev, 1996
Alsophylax pipiens	42	9.5	6.5	16	1.5	38.1	0.391	Szczerbak and Golubev, 1996
Alsophylax tadjikiensis	31	8.5	5.6	14.1	1.5	45.5	0.472	Szczerbak and Golubev, 1996
Alsophylax tokobajevi	46	12	7.9	19.4	1.5	42.2	0.436	Szczerbak and Golubev, 1996
Aristelliger barbouri	46	11.8	8.0	19.8	1.7	36.8	0.377	Schwartz and Henderson, 1991
Aristelliger cochranae	53	11.1	8.4	19.5	1.3	32.4	0.33	Schwartz and Henderson, 1991
Aristelliger praesignis	78	14	11	25.3	1.2	32.4	0.33	Grant, 1940
Asaccus elisae	70	15	10	25	1.5	36	0.368	Leptien et al., 1994a; Leptien, 1996
Asaccus gallagheri	40	9	6	15	1.5	37.5	0.384	Leptien et al., 1994b; Leptien, 1996
Bunopus blanfordii	53	13	8.7	21.7	1.5	40.9	0.421	Werner, 1989
Bunopus spatalurus	45	10	8	18	1.3	40.0	0.412	Leptien and Zilger, 1991
Bunopus tuberculatus	53	10	8	18	1.3	34.0	0.347	Leptien, 1993
Calodactylodes illingworthi	84	14	12	26	1.2	31.0	0.315	Deraniyagala, 1953
Chondrodactylus angulifer	102	21	15	35.5	1.4	34.8	0.355	Henkel, F. D., 1992

Table A7.1 Gekkonines (continued)

Christinus guentheri	100	14	13	27	1.1	27.0	0.273	Cogger, Sadlier and Cameron, 1983.
Christinus marmoratus	68	13	12	24.1	1.1	35.4	0.362	Bustard, 1965a; King, 1977; Armstrong, 1979
Cnemaspis africana	54	9	7.5	16.5	1.2	30.6	0.311	Loveridge, 1947
Cnemaspis elgonensis	61	11	9.5	20	1.1	32.8	0.334	Loveridge, 1942c; Loveridge, 1947
Cnemaspis jerdonii	35	4.7	4	8.7	1.2	24.9	0.252	Deraniyagala, 1953
Cnemaspis kandiana	31	5.5	4.5	10	1.2	32.3	0.329	Deraniyagala, 1953
Cnemaspis koehleri	54	9	7	16	1.3	29.6	0.301	Loveridge, 1947
Cnemaspis podihuna	26	5.5	4.7	10.2	1.2	39.2	0.403	Deraniyagala, 1953
Cnemaspis quattuorseriatus	45	7	6	13	1.2	28.9	0.293	Loveridge, 1942b; Loveridge, 1947
Cnemaspis tropidogaster	31	5.5	4.5	10	1.2	32.3	0.329	Deraniyagala, 1953
Cosymbotus platyurus	58	11	8.9	19.9	1.2	34.3	0.35	Chou,1979
Crossobamon eversmanni	59	12	8.9	20.6	1.3	34.9	0.357	Szczerbak and Golubev, 1996
Cyrtodactylus amictopholis	34	10.3	6.6	16.9	1.6	49.7	0.52	Werner, 1989
Cyrtodactylus baluensis	86	15.4	12.4	27.8	1.2	32.3	0.329	Hikida, 1990
Cyrtodactylus consobrinus	121	15	12	27.8	1.2	23.0	0.232	Hikida, 1990
Cyrtodactylus fraenatus	100	17	14	31.3	1.2	31.3	0.318	Deraniyagala, 1953
Cyrtodactylus louisiadensis	160	19	16	34.7	1.2	21.7	0.219	T. Annable, unpublished findings
Cyrtodactylus peguensis	70	11	9	20	1.2	28.6	0.29	Henkel and Schmidt, 1995
Cyrtopodion elongatus	45	7	5.5	12.5	1.3	27.8	0.282	Wai, 1994
Cyrtopodion kachhensis	42	9.5	7	16.5	1.4	39.3	0.404	Minton, 1966; Szczerbak and Golubev, 1996
Cyrtopodion scaber	53	10.5	7	17.5	1.5	33.0	0.336	Szczerbak and Golubev, 1996
Dravidogecko anamallensis	54.4	9.3	7.9	17.2	1.2	31.6	0.322	Gvosdik and Vesely, 1998
Geckoella triedrus	51	12	10	22	1.2	43.1	0.446	Deraniyagala, 1953
Geckolepis typica	80	14	12	26	1.2	32.5	0.331	Henkel and Schmidt, 1995
Geckonia chazaliae	57	13	10	23	1.3	40.4	0.416	Henkel and Schmidt, 1995
Gehyra australis	70	13	10	22.8	1.2	32.6	0.332	Bustard, 1969
Gehyra dubia	70	11	10	21	1.1	30.0	0.305	Henkel and Schmidt, 1995
Gehyra mutilata	51	5	4.5	9.5	1.1	18.6	0.187	McCann, 1955
Gehyra oceanica	67	13	12	24.4	1.1	36.4	0.373	Schwaner, 1980;
Gehyra variegata	55	11	9.3	20.1	1.2	36.5	0.374	Bustard, 1968a; T. Annable, unpublished findings

Table A7.1 Gekkonines (continued)

Gehyra vorax	152	20	18	38	1.1	25.0	0.253	Boulenger, 1885; Gibbons and Zug, 1987
Gekko gecko	178	20	18	38	1.1	21.3	0.215	Bauer and Russell, 1991; Daniel, 1983; Henkel and Schmidt, 1995
Gekko monarchus	95	13.5	10	23.5	1.4	24.7	0.25	Boulenger, 1912; Rogner, 1997
Gekko petricolus	75	12	10	22	1.2	29.3	0.297	Taylor, 1962; Rogner, 1997
Gekko smithi	190	24	22	46	1.1	24.2	0.244	Rogner, 1997Gymnodactylus frenatus
Gymnodactylus frenatus	100	16.8	14.5	31.3	1.2	31.3	0.318	Deraniyagala, 1953
Gymnodactylus geckoides	49	9.3	6.1	15.4	1.5	31.4	0.319	Vitt, 1968
Hemidactylus bowringii	41.1	8.7	7.6	16.3	1.1	39.7	0.408	Ota and Lin, 1997
Hemidactylus brookii	52	8.6	7.5	16.1	1.1	31.0	0.315	Loveridge, 1947; Deraniyagala, 1953
Hemidactylus flaviviridis	90	13	11	23	1.2	25.6	0.259	Loveridge, 1947; Daniel, 1983
Hemidactylus frenatus	81	10	7.6	17.7	1.3	21.9	0.221	Loveridge, 1947; Minton, 1966; Schwaner, 1980
Hemidactylus garnotii	60	10	9	19	1.1	31.9	0.325	Gibbons and Zug, 1987
Hemidactylus leschenaultii	80	11	10	21	1.1	26.2	0.265	Boulenger, 1912; Rogner, 1997
Hemidactylus mabouia	86	12	9	20.5	1.3	23.8	0.24	Loveridge, 1947; Meshaka et al., 1994.
Hemidactylus maculatus	135	19	16	35	1.2	25.9	0.262	Daniel, 1983
Hemidactylus mercatorius	52	8.8	8.5	17.3	1.0	33.3	0.339	Loveridge, 1942a; Loveridge, 1961
Hemidactylus squamulatus	48	9	8.3	17.3	1.1	36.0	0.368	Loveridge, 1947
Hemidactylus triedrus	81	12	10	21.8	1.2	26.9	0.272	Deraniyagala, 1953
Hemidactylus turcicus	59	12	10	21.5	1.2	36.4	0.373	Loveridge, 1947; Minton, 1966; Seufer, 1991; Cloudsley-Thompson, 1995
Hemidactylus yerburii	59	11	9.3	19.8	1.1	33.6	0.343	Lanza, 1978
Hemiphyllodactylus typus	60	8	6	14	1.3	23.3	0.235	Vinson and Vinson, 1969; Daniel, 1983
Heteronotia binoei	80	9.9	7.5	17.4	1.3	21.8	0.22	T. Annable, unpublished findings
Homopholis boivini	150	25	15	40	1.7	26.7	0.27	Dewitt, 1996
Lepidodactylus euaensis	50	11	7.5	18.5	1.5	37.0	0.379	Gibbons and Brown, 1988
Lepidodactylus intermedius	42	6.9	6.5	13.4	1.1	31.9	0.325	Parker, 1926; Darevsky, 1964
Lepidodactylus lugubris	48	8.7	7	15.7	1.2	32.7	0.333	Deraniyagala, 1953; Schwaner, 1980
Lepidodactylus vanuatuensis	47.4	9.5	6.5	16	1.5	33.8	0.345	Ota, Fisher, Ineich, Case, Radtkey and Zug, 1998
Lygodactylus angularis	46	8	6.8	14.8	1.2	32.2	0.328	Loveridge, 1947; Loveridge, 1953
Lygodactylus capensis	31	7	5	12	1.4	38.7	0.397	Broadley, 1971
Lygodactylus grotei	33	6.3	5.5	11.8	1.1	35.8	0.366	Loveridge, 1947
Lygodactylus gutturalis	38	8	6	14	1.3	36.8	0.377	Loveridge, 1947

Table A7.1 Gekkonines (continued)

Lygodactylus klugei	34	6.6	4.5	11.1	1.5	35.8	0.366	Vitt, 1986
Lygodactylus picturatus	37	7	5.5	12.5	1.3	33.8	0.345	Loveridge, 1947; Henkel and Schmidt, 1995
Mediodactylus kotschyi	56	11	9.0	20	1.2	35.7	0.365	Seufer, 1991; Loveridge, 1947; Werner, 1989; Szczerbak and Golubev, 1996
Nactus pelagicus	69	13	7	20	1.9	29.0	0.294	Gibbons and Zug, 1987
Narudasia festiva	31	7.6	6	13.6	1.3	43.9	0.454	Loveridge, 1947
Pachydactylus bibronii	98	14.4	13.1	27.5	1.1	28.1	0.285	De Waal, 1978; Douglas, 1990, Seufer, 1991; Henkel and Schmidt, 1995
Pachydactylus bicolor	43	9.2	6	15.2	1.5	35.3	0.361	Loveridge, 1947
Pachydactylus capensis	53	11	8	19	1.4	35.8	0.366	Loveridge, 1947
Pachydactylus geitje	40	9.5	7	16.5	1.4	41.3	0.426	Loveridge, 1947; Bustard, 1963
Pachydactylus maculatus	45	9	8	17	1.1	37.8	0.388	Loveridge, 1947
Pachydactylus namaquensis	82	16	12	28	1.3	34.1	0.348	Loveridge, 1947
Pachydactylus punctatus	40	8.3	6.6	14.9	1.3	37.3	0.382	Loveridge, 1947
Pachydactylus scutatus	36	7.2	5.5	12.7	1.3	35.3	0.361	Loveridge, 1947
Pachydactylus weberi	42	10	6.5	16.5	1.5	39.3	0.404	Loveridge, 1947
Palmatogecko rangei	80	13	9.5	22	1.3	27.5	0.279	Furman, 1994
Phelsuma astriata	52	9.7	8.0	17.7	1.2	34.0	0.347	Bourquin and Hitchins, 1998
Phelsuma borbonica	55	12	10	22	1.2	40.0	0.412	Girard, 1995
Phelsuma cepediana	49	10	8	18	1.3	36.7	0.376	Vinson and Vinson, 1969
Phelsuma guentheri	120	20	18	38	1.1	31.7	0.323	Vinson and Vinson, 1969; Garbutt, 1993
Phelsuma klemmeri	40	6	6	12	1.0	30.0	0.305	Henkel and Schmidt, 1995
Phelsuma madagascariensis	145	15.5	15.5	31	1.0	21.4	0.216	Henkel and Schmidt, 1995; R. Porter, personal communication
Phelsuma ocellata	40	8	6.5	14.5	1.2	36.3	0.371	Knothig, 1994
Phelsuma standingi	133	18	17	35	1.1	26.3	0.266	Zaworski, 1992; Voorhees, 1993; Hoskisson, 1995
Phelsuma vinsoni	48	10	9	19	1.1	39.6	0.407	Vinson and Vinson, 1969
Phyllodactylus lineatus	30	7.8	6.4	14.2	1.2	47.3	0.493	Loveridge, 1947
Phyllodactylus porphyreus	51	11	8	18.5	1.3	36.3	0.371	Loveridge, 1947
Phyllopezus pollicaris	85	13.1	9.4	22.5	1.4	26.5	0.268	Vitt, 1986
Pristurus flavipunctatus	35	7	6	13	1.2	37.1	0.38	Loveridge, 1947
Ptyodactylus hasselquistii	90	14	13.1	27.1	1.1	30.1	0.306	Werner, 1986, 1989; Schleich and Kästle, 1988
Rhoptropus barnardi	44	12	9.5	21	1.2	47.7	0.497	Loveridge, 1947

Table A7.1 Gekkonines (continued)

*Stenodactylus arabicus**	40	9	4	13	2.3	32.5	0.331	Leptien, 1992
Stenodactylus doriae	67	12.3	10.5	22.8	1.2	34.0	0.347	Werner, 1989
Stenodactylus sthenodactylus	55	11.2	9.2	20.4	1.2	37.1	0.38	Werner, 1989
Tarentola americana	111	14.1	11.8	25.9	1.2	23.3	0.235	Schwart and Henderson, 1991
Tarentola annularis	140	15	10	25	1.5	17.9	0.18	Zaworski, 1992
Tarentola delalandii	65.5	12	9.5	21.5	1.3	32.8	0.334	Joger, 1984; Schleich and Kästle, 1988
Tarentola gigas	127	20	20	40	1.0	31.5	0.32	Schleich and Kästle, 1988
Tarentola mauritanica	80	13	10	23	1.3	28.8	0.292	Loveridge, 1947
Tenuidactylus caspius	56	11	9.1	20.2	1.2	36.1	0.369	Szczerbak and Golubev, 1996
Tenuidactylus fedtschenkoi	70	12	9.5	21.3	1.2	30.4	0.309	Szczerbak and Golubev, 1996
Tenuidactylus russowii	41	11	8.2	18.7	1.3	45.6	0.473	Szczerbak and Golubev, 1996
Tenuidactylus spinicauda	48	9.1	7.4	16.5	1.2	34.4	0.351	Szczerbak and Golubev, 1996
Tenuidactylus turcmenicus	71	13	8.8	21.5	1.4	30.3	0.308	Szczerbak and Golubev, 1996
Teratolepis fasciata	67	9.8	9	18.8	1.1	28.1	0.285	Minton, 1966; Girard, 1993;
Teratoscincus microlepis	77	18	16	34	1.1	44.2	0.458	Seufer, 1991; Girard, 1993; Szczerbak and Golubev, 1996;
Teratoscincus przewalskii	77	17	16	32	1.1	41.6	0.429	Szczerbak and Golubev, 1996
Teratoscincus roborowskii	76	18	17	35	1.1	46.1	0.479	Wai, 1994a
Teratoscincus scincus	81.8	19	17	35.8	1.1	43.8	0.453	Minton, 1966; Szczerbak and Golubev, 1996
Tropiocolotes helenae	32	8	5.5	13.5	1.5	42.2	0.436	Minton, 1966
Tropiocolotes levitoni	45	13	9	22	1.4	48.9	0.511	Szczerbak and Golubev, 1996
Tropiocolotes persicus	36	8	5.7	13.7	1.4	38.1	0.391	Szczerbak and Golubev, 1996
Tropiocolotes steudneri	27	7	5.4	12.4	1.3	44.3	0.459	Werner, 1989
Uroplatus ebenaui	40	5	5	10	1.0	25.0	0.253	Henkel and Schmidt, 1995
Uroplatus fimbriatus	186	20	20	40	1.0	21.5	0.217	Henkel, F. D., 1992
Uroplatus guentheri	80	7	6.5	13.5	1.1	16.9	0.17	Henkel, F. D., 1992; Henkel and Schmidt, 1995
Uroplatus henkeli	145	18	18	36	1.0	24.8	0.251	Henkel, F. D., 1992

* Egg measurements probably in error

Table A7.1 (continued)
SPHAERODACTYLINE GECKOS

Gonatodes albogularis	50	8	6.5	14.5	1.2	29.0	0.294	Grant, 1940
Gonatodes vittatus	43	7.8	6.3	14.1	1.2	32.8	0.334	Garman, 1887
Lepidoblepharis intermedius	29	6.9	6.5	13.4	1.06	46.2	0.48	Boulenger, 1914; Parker, 1926
Lepidoblepharis sanctaemartae	21.5	3.5	3.0	6.5	1.2	30.2	0.307	Ruthven, 1916; Seufer, 1991; Markezich and Taphorn, 1994
Sphaerodactylus altavelensis	29	5.8	4.2	10.2	1.4	35.2	0.36	Schwartz and Henderson, 1991
Sphaerodactylus argus	33	8.0	5.8	13.8	1.4	41.8	0.431	Schwartz and Henderson, 1991
Sphaerodactylus asterulus	31	6.6	5.4	12.0	1.2	38.7	0.397	Schwartz and Henderson, 1991
Sphaerodactylus caicosensis	32	7.7	5.7	13.4	1.4	41.9	0.432	Schwartz and Henderson, 1991
Sphaerodactylus copei	40	9.3	7.3	16.6	1.3	41.5	0.428	Schwartz and Henderson, 1991
Sphaerodactylus corticola	39	8.0	6.1	14.1	1.3	36.2	0.37	Schwartz and Henderson, 1991
Sphaerodactylus darlingtoni	20	6	4	10	1.5	50.0	0.524	Schwartz and Henderson, 1991; Henkel and Schmidt, 1995
Sphaerodactylus difficilis	25	8	6	14	1.3	56.0	0.594	Schwartz and Henderson, 1991; Henkel and Schmidt, 1995
Sphaerodactylus elegans	37	8.0	5.9	13.9	1.4	37.6	0.385	Schwartz and Henderson, 1991
Sphaerodactylus elegantulus	29	7.0	5.5	12.5	1.3	43.1	0.446	Schwartz and Henderson, 1991
Sphaerodactylus fantasticus	29	7.0	4.9	11.8	1.4	40.7	0.419	Schwartz and Henderson, 1991
Sphaerodactylus goniorhynchus	25	4.5	3.5	8.0	1.3	32.0	0.326	Schwartz and Henderson, 1991
Sphaerodactylus inaguae	28	7.0	5.0	12.0	1.4	42.9	0.443	Schwartz and Henderson, 1991
Sphaerodactylus klauberi	37	7.6	5.6	13.1	1.4	35.4	0.362	Schwartz and Henderson, 1991
Sphaerodactylus macrolepis	31	6.3	5	11.3	1.3	36.5	0.374	Garman1887; Schwartz and Henderson, 1991
Sphaerodactylus mariguanae	41	7.4	5.8	13.2	1.3	32.2	0.328	Schwartz and Henderson, 1991
Sphaerodactylus microlepis	34	7.1	5.7	12.8	1.3	37.6	0.385	Schwartz and Henderson, 1991
Sphaerodactylus micropithecus	32	9.2	6.3	15.5	1.5	48.4	0.505	Schwartz and Henderson, 1991
Sphaerodactylus nigropunctatus	40	7.8	6.1	13.9	1.3	34.8	0.355	Schwartz and Henderson, 1991
Sphaerodactylus notatus	34	6.9	5.1	12.0	1.4	35.3	0.361	Schwartz and Henderson, 1991
Sphaerodactylus randi	32	7.6	5.1	12.7	1.5	39.7	0.408	Schwartz and Henderson, 1991
Sphaerodactylus roosevelti	38	8.5	6.7	15.2	1.3	40.0	0.412	Thomas and Schwartz, 1966
Sphaerodactylus savagei	33	7.3	5.0	12.3	1.5	37.3	0.382	Schwartz and Henderson, 1991

Table A7.1 (continued)
DIPLODACTYLINE GECKOS

Carphodactylus laevis	130	25.5	14.1	39.6	1.81	30.5	0.31	Ehmann, 1992; R. Porter, personal communication
Diplodactylus galeatus	54	15.1	7.7	22.8	1.96	42	0.43	Ehmann, 1992; G. Husband, personal communication
Eurydactylodes vieillardi	65	13.3	8.3	21.6	1.60	29.6	0.3	R. Porter, personal communication
Nephrurus amyae	135	30.8	17.3	48.1	1.78	35.8	0.37	T. Annable, unpublished findings
Nephrurus asper	118	26.9	15.5	42.4	1.74	35.9	0.37	T. Annable, unpublished findings
Nephrurus deleani	89	23.5	12.8	36.3	1.84	40.8	0.42	T. Annable, unpublished findings
Nephrurus laevissimus	95	19.5	10.1	29.6	1.93	31.2	0.32	N. Sonnemann, personal communication
Nephrurus levis	91	24.5	13.9	38.3	1.78	42.1	0.43	T. Annable, unpublished findings
Nephrurus sheai	121	29.5	16	45.5	1.84	37.6	0.39	Gow, 1979
Nephrurus stellatus	86	22.4	12.5	34.9	1.79	40.6	0.42	R. Porter, personal communication
Oedura castelnaui	97	22.4	10.7	33.1	2.09	34.1	0.35	Bustard, 1967c
Oedura coggeri	70	19.1	9.9	29	1.93	41.4	0.43	R. Porter, personal communication
Oedura filicipoda	105	24.3	11.9	36.1	2.04	34.4	0.35	R. Porter, personal communication
Oedura gemmata	100	23.8	11.8	35.5	2.02	35.5	0.36	R. Porter, personal communication
Oedura lesueurii	67	16	7.8	23.8	2.05	35.5	0.36	Bustard, 1967c
Oedura marmoratus	100	25.3	10.4	35.7	2.43	35.7	0.37	Bustard, 1967c
Oedura monilis	92	23	10.9	33.9	2.11	36.8	0.38	Bustard, 1967c; Rösler, 1980
Oedura tryoni	90	20.1	11.1	30.2	1.81	33.6	0.34	Bustard, 1967; Porter, personal communication
Phyllurus caudiannulatus	86.5	20	11	31	1.82	35.8	0.37	R. Porter, personal communication
Phyllurus championae	80.6	18	9.0	27	2.00	33.5	0.34	Couper et al., 2000
Phyllurus platurus	96	23.5	12.1	35.6	1.94	37.1	0.38	R. Porter, personal communication
Pseudothecadactylus lindneri	100	28	15	43	1.86	43.0	0.44	Sonnemann, 1998
Rhacodactylus auriculatus	120	25	15	40	1.67	33.3	0.34	Henkel, F. W., 1991; Henkel, F.W., 1993
Rhacodactylus chahoua	167	30	14.5	44.5	2.07	26.6	0.27	Henkel, F. W., 1991; Henkel, F. W., 1993
Rhacodactylus ciliatus	109	21	13.1	34.1	1.60	31.3	0.32	Seipp and Klemmer, 1994
Rhacodactylus leachianus	245	36.5	21	57.5	1.74	23.5	0.24	T. Annable, unpublished findings

Table A7.1 Diplodactylines (continued)

Rhacodactylus sarasinorum	135	28	11	39	2.5	28.9	0.293	Henkel, F. W. 1987; Henkel, F. W., 1993
Saltuarius cornutus	140	25.4	15.2	40.6	1.7	29.0	0.294	R. Porter, personal communication
Saltuarius salebrosus	133	29	18.2	47.2	1.6	35.5	0.363	R. Porter, personal communication
Saltuarius swaini	130	24.1	13.7	37.8	1.8	29.1	0.295	R. Porter, personal communication
Saltuarius wyberba	109	24.7	15.1	39.8	1.6	36.5	0.374	R. Porter, personal communication
Diplodactylus ciliaris	89	15.6	8.9	24.4	1.75	27.4	0.28	Husband, 1998; Porter ,personal communication
Diplodactylus elderi	45	13.3	8.5	21.8	1.56	48.4	0.51	Bustard, 1965a
Diplodactylus taenicauda	73	16.2	8.9	25.1	1.82	34.4	0.35	Ehmann, 1992; R. Porter, personal communication
Strophurus williamsi	65	15.6	9.6	25.2	1.6	38.8	0.398	Shea, 1984
Underwoodisaurus milii	110	23.7	12.2	35.9	1.9	35.2	0.36	T. Annable, unpublished findings
Underwoodisaurus sphyrurus	86	22	11	33	2.0	38.8	0.398	T. Annable, unpublished findings
EUBLEPHARINE GECKOS								
Aeluroscalabotes felinus	110	19.2	11.1	30.3	1.7	27.5	0.279	Werner, 1972; Nunan, 1994
Coleonyx variegatus	80	19	9	28	2.1	35.0	0.358	Henkel and Schmidt, 1995
Eublepharis macularius	127	28	15	43	1.9	33.9	0.346	Minton, 1966; Daniel, 1983; Henkel and Schmidt, 1995; Szczerbak and Golubev, 1996
Eublepharis turcmenicus	135	35	19	54.2	1.8	40.1	0.413	Szczerbak and Golubev, 1996
Goniurosaurus kuroiwae	82	20	12	32	1.7	39.0	0.401	Henkel and Schmidt, 1995
Hemitheconyx caudicinctus	120	27	13	40	2.1	33.3	0.339	Werner, 1972; Seufer, 1991; Anderson, 1993

Table A7.1 (continued)

SUBTOTALS	SVL	L	W	L+W	L/W	L+W*100/ SVL
Rigid shell species max.	190	25	20	46	2.3	56.0
Rigid shell species min.	20	3.5	3	6.5	1	16.9
Rigid shell species average	62.1	10.9	8.8	19.7	1.27	34.2
Rigid shell species STD	35.2	4.15	3.87	7.89	0.18	7.08
Rigid shell species N	162	162	162	162	162	162
Flexible shell species max.	245	37	21	57.5	2.5	48.4
Flexible shell species min.	45	13	7.8	21.6	1.6	23.5
Flexible shell species average	104	23.2	12.5	35.5	1.87	35.1
Flexible shell species STD	33.8	5.5	3.1	8.4	0.21	5.0
Flexible shell species N	43	43	43	43	43	43
TOTALS (all geckos)						
Maximum	245	37	22	57.5	2.5	56.0
Minimum	20	3.5	3	6.5	1	16.9
Average	70.9	13.5	9.58	23.0	1.40	34.4
STD	38.8	6.69	4.00	10.3	0.30	6.70
N	205	205	205	205	205	205

Table A7.2 *Clutch sizes of gecko species.*

All sphaerodactyline species lay a single egg in a clutch (Miller, 1984; Schwartz and Henderson, 1991).

All diplodactyline and eublepharine species usually lay two eggs in a clutch (Greer, 1989).

Some gekkonine species lay only one egg, while most lay two eggs per clutch (Greer, 1989).

Species Laying Only One Egg/ Clutch	**Reference**
Aristelliger barbouri	Hecht, 1952
Aristelliger cochranae	Hecht, 1952
Aristelliger expectatus	Hecht, 1952
Aristelliger georgeensis	Hecht, 1952
Aristelliger hechti	Hecht, 1952
Aristelliger lar	Hecht, 1952
Aristelliger praesignis	Hecht, 1952
Asaccus caudivolvulus	Arnold and Gardner, 1994
Asaccus elisae	Arnold and Gardner, 1994
Asaccus gallagheri	Arnold and Gardner, 1994
Asaccus griseonotus	Arnold and Gardner, 1994
Asaccus montanus	Arnold and Gardner, 1994
Asaccus platyrhynchus	Arnold and Gardner, 1994
Hemidactylus bowringii	Ota and Lin, 1997
Homonota fasciatus (locally)	Lavilla, Cruz, and Scrocchi, 1995.
Gehyra variegata	Bustard, 1968a; Greer, 1989; Cronin, 2001
Saurodactylus mauritanicus	Henkel and Schmidt, 1995
Stenodactylus arabicus	Leptien, 1992
Thecadactylus rapicauda	Vitt and Zani, 1997
Tropiocolotes tripolitanus	Henkel and Schmidt, 1995

Table A7.3 Gekkonine Species Laying Either One or Two Eggs per Clutch

Species	**Reference**
Alsophylax laevis	Seufer, 1991
Alsophylax loricatus	Szczerbak and Golubev, 1996
Alsophylax tadjikiensis	Szczerbak and Golubev, 1996
Asiocolotes levitoni	Golubev and Szczerbak 1979
Bunopus spatalurus	Arnold, 1984
Bunopus tuberculatus	Minton, 1966; Szczerbak and Golubev, 1996
Christinus alexanderi	Donnellan, Aplin and Dempsey, 2000
Christinus guentheri	Ehmann, 1992
Christinus marmoratus	Thompson and Russell, 1999; Cronin, 2001
Cnemaspis kandiana	Deraniyagagala, 1953
Cnemaspis tropidogaster	Deraniyagagala, 1953
Crossobamon eversmanni	Seufer, 1991
Cyrtodactylus louisiadensis	Cronin, 2001
Cyrtopodion kachhensis	Minton, 1966
Gekko petricolus	Rogner, 1997
Gymnodactylus geckoides amarali	Colli et al., 2003
Hemidactylus brookii	Calderon et al., 1994
Hemidactylus flaviviridis	Arnold, 1984
Hemidactylus turcicus	Stebbins, 1948
Homonota fasciata	Lavilla, Cruz and Scrocchi, 1995
Lepidodactylus lugubris	Henkel and Schmidt, 1995
Phelsuma madagascariensis grandis	Henkel and Schmidt, 1995
Phelsuma standingi	Voorhees, 1993
Phyllodactylus xanti	Stebbins, 1948
Mediodactylus kotschyi	Seufer, 1991
Mediodactylus russowii	Szczerbak and Golubev, 1996
Mediodactylus spinicauda	Szczerbak and Golubev, 1996
Stenodactylus khobarensis	Arnold, 1984

APPENDIX 8

ACKNOWLEDGEMENTS

(in alphabetic order)

Lloyd Allen and Christopher Annable for much appreciated assistance with histology.

My wife Joanne Annable for her patience with my impatience.

Susan Annable for assistance with proof-reading.

Wendy Annable for help with proof reading.

Ken Aplin, for *Nephrurus* and *Underwoodisaurus* locality data and loan of museum specimens.

Maurice Ashton, for assistance with photo-scanning and geographical distribution plotting.

Aaron Bauer, for providing an avalanche of 'gekkonoid' literature and his general encouragement with my project.

Terry Boylan, for general gecko information.

Brian Bush, for *Nephrurus* locality information.

Ken Chapman, for building palatial vivariums for the geckos.

Hal Cogger, for gecko information and invaluable reference sources.

Ray Cottier for very considerable assistance with electrical arrangements for experimentation and advice on circuitry, and building 'in-floor' heating units.

Jeanette Covacevich, for triggering my initial interest in geckos.

Matt Devine, first field trip to South Australia.

Chris Dickman and Rebecca Drury, for specimens of *N. levis*.

Bob Drewer for permission and encouragement to undertake the research.

Ina Fine, for tremendous assistance in interlibrary loans and literature searching.

Greg Fyfe, for *Nephrurus* locality data and provision of *N. amyae* specimens.

Carl Gans, for herpetological support, advice and generous donation of two herpetology textbooks..

Allen Greer, for herpetological inspiration and general *Nephrurus* information.

Bill Hartley, for advice on gecko pathology.

Bevan Hokin for allowing the use of the pathology laboratory facilities at Sydney Adventist Hospital.

Paul Horner, for *Nephrurus* locality data and loan of museum specimens.

David Howse, for assistance with much of the histology.

Mark Hutchinson, for *Nephrurus* locality data and loan of museum specimens.

Peter Jones, for additional *Nephrurus* locality data.

Tom Joys, for assistance with photomicrography.

Ian Kaplin, for assistance with X-Ray analysis of gecko eggshells.

Rie Kawashima, for translations from Japanese.

James King for assistance with radiography of specimens.

Eva Klein, for translations from German.

Danielle Lee, for assistance in final typing and formatting of the thesis.

Geof Madigan, for general support and recommendation for Advanced Study funding.

Jason Morton for assistance with my 'other' work.

Liliana Munoz for feeding the geckos while I was away on field trips.

Peter Page for *Nephrurus* egg data.

Denise Palmer for supply of *Underwoodisaurus* specimens.

Rob Porter for general gecko information and advice on geckos also provision of egg data and loan of specimens.

Tony Romeo, Francis Chee & Ann for great help with SEM.

Patrick Couper, for locality data and loan of museum specimens,

Kylie Russel, for technical assistance when microbombing.

Anthony Russell, for even more 'Gekkonoid' literature.

Ross Sadlier, for locality data and loan of museum specimens.

Laurie Smith, for locality information on *N. wheeleri* and loan of museum specimens.

Neil Sonnemann, for gecko egg data.

Naz Soran for technical assistance with the Kjeldahl apparatus.

Phil Spark, for locality and habitat information on *U. sphyrurus*.

Mike Speak, for invaluable assistance and advice on electron microscopy and scanning images.

Ann Stafford for help with colour processing and binding.

Gerry Swan, for general gecko information and locality advice.

Mike Thompson, for technical support, great patience and general encouragement.

Brian Timms for assistance with proof reading.

Dale Val and Dean Valderemo for field trip transport and assistance.

Lyndon Voigt for taking an increased workload.

Adrian Wayne, for gecko specimens and information on the biology of *N. stellatus*.

Ivan Wittwer, for provision of locality data for *N. stellatus*.

John Wombey, for *Nephrurus* locality data and loan of specimens.

Also, the Avondale Foundation, which provided financial assistance for several field trips which triggered this research project.

Conservation Foot-note

Apart from some eggs and embryos no specimens were euthanased specifically for this study.