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September 1998
The female reproductive cycle of a viviparous skink, *Oligosoma maccanni*, in a subalpine environment

Karina Holmes

A thesis submitted in partial fulfilment for

the degree of Master of Science

at the University of Otago, Dunedin,

New Zealand

25 March 2004
ABSTRACT

The majority of New Zealand’s endemic reptiles that have been studied with respect to patterns of reproduction show low rates of annual reproductive output, with extended gestation and great longevity. A well-studied example of extended gestation is the common gecko (*Hoplodactylus maculatus*) at the subalpine site of Macraes Flat. At this location, females have biennial reproduction with a gestation of up to 14 months, while at a warmer site gestation only lasts 3-5 months. The main aim of this thesis was to investigate whether McCann’s skink (*Oligosoma maccanni*) also has extended gestation at the same subalpine site. Relative clutch mass (RCM) was compared between the two species to investigate possible differences of maternal investment in reproduction. Palpation was used to assess reproductive condition of *O. maccanni* non-invasively, and a subset of the sample was dissected to confirm reproductive condition and to test the accuracy of palpation. Both species have autumn-spring vitellogenesis; however, in contrast to *H. maculatus*, female *O. maccanni* are annual breeders with a spring-summer gestation of 4-5 months. I hypothesised that RCM would be smaller in *O. maccanni* in comparison to *H. maculatus* as a trade-off to allow annual reproduction. However, a lack of overlap in body size constrained comparisons of RCM between the two species. I suggest that if an overlap did occur it is unlikely that *O. maccanni* have a smaller RCM, and the difference in reproductive cycles is more likely due to other factors. Palpation was accurate in distinguishing vitellogenic, early- to mid-pregnant and late-pregnant females from each other. It was also highly accurate in estimating clutch size in females in early-mid pregnancy, but not as accurate in vitellogenic or late-pregnant females. This study has provided important information for evolution and life history studies and for the conservation of *O. maccanni* and other New Zealand lizards.
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CHAPTER ONE

General Introduction

1.1 Reproduction in reptiles

In order to understand what limits a species' distribution and reproductive output, one must first understand the life history of the species, especially in terms of annual or geographic variation (Wapstra and Swain 2001). Reptiles exhibit a vast diversity of life histories, and comparisons among and within species assist in understanding life history evolution. Reproductive characteristics that differ among species include: parity mode (oviparous [egg-laying] or viviparous [live-bearing]); size and number of eggs or embryos; rate of embryonic development; frequency of reproduction; and timing of reproduction. In order to understand this variation, a description of the stages and events in a reproductive cycle is required.

1.2 Stages in the female reproductive cycle of viviparous lizards

Reproduction is a series of physiological processes that allow an individual to reproduce. In females of viviparous species, these stages are vitellogenesis, ovulation, gestation and parturition. Vitellogenesis is the process in which the yolk protein precursor (vitellogenin) is synthesized in the liver and released into the circulatory system, from which it is taken up by the developing oocytes in the ovary (Ho 1987, 1991). In most species the resulting yolk provides most of the nutrients for the developing embryo (lecithotrophy) (Weekes 1935; Yaron 1985; Stewart 1992). However, there are some species of reptiles in which most nutrients are transferred from the mother through a placenta (placentotrophy), and yolk is
fertilization occurs circulating levels increase significantly, usually peaking around the second trimester and then declining prior to parturition (Bourne et al. 1986; Callard et al. 1992; van Wyk 1994; Jones and Swain 1996; Jones et al. 1997). The specific role of progesterone in gestation in viviparous squamates remains unclear. Some ideas for the role of progesterone include slowing the rate of ovarian development (Jones and Baxter 1991; Callard et al. 1992; Bonnet et al. 2001), influencing the rate of embryonic development (Gemmel 1995), and preparing for and/or maintaining gravidity (Yaron 1985; Guillette et al. 1991; Jones and Baxter 1991; Girling 2002).

1.4 Cycles in lizards of northern and southern hemispheres

Many female lizards from temperate environments in the northern hemisphere concentrate their reproductive activity during the warmer months of the year so that vitellogenesis, gestation and parturition occur during spring and summer. Gonads are generally quiescent during autumn and winter in these species (Fitch 1970; Duvall et al. 1982). Many species in the southern hemisphere also give birth during spring or summer (and occasionally early autumn). However, they seem to have reproductive activity extending throughout most of the year. This is observed in many female viviparous lizards from temperate locations in the southern hemisphere (New Zealand, Tasmania [Australia], South Africa and South America), where vitellogenesis occurs during autumn-spring, gestation occurs during spring and summer, and gonads are quiescent for only a short time during late summer or early autumn (van Wyk 1991; Cree 1994; Jones and Swain 1996; Jones et al. 1997; Ibargüengoytia and Cussac 1998; Wilson and Cree 2003). In some species that have this extended reproduction throughout the year, occasional individuals skip a year of reproduction. In such instances, the gonads remain quiescent until the next vitellogenic cycle the following year (van Wyk 1991; Ibargüengoytia and Cussac 1996).
reduced in these species (van Wyk 1994; Blackburn 2000; Ramírez-Pinilla et al. 2002).

Once oocytes have matured and reached a species-specific size, they are ovulated (rupture from the ovary) and pass into the oviduct where they can be fertilized. The cells of the ovulated follicles that are left behind in the ovary proliferate and luteinize to form corpora lutea (Norris 1997). If fertilization occurs, the corpora lutea persist for a species-specific amount of time and secrete progesterone, a sex-steroid hormone. The gestation period is the time from fertilization till parturition (birth). In viviparous species, therefore, all embryonic development occurs during the gestation period. Timing of parturition may be influenced by the strong temperature dependence of embryogenesis (Olsson and Shine 1998). Therefore, reptiles from cool climates (high latitude and/or altitude) are likely to have delayed parturition relative to conspecifics from warmer regions (Ibargüengoytía and Cussac 1998; Olsson and Shine 1998).

1.3 Plasma hormone profiles

In females of seasonally cycling lizards, plasma concentrations of progesterone and estradiol vary throughout the year, suggesting roles for each steroid in the regulation of various stages of the annual reproductive cycle (Edwards and Jones 2001). Most viviparous lizards from temperate zones show similar cycles in estradiol and in progesterone (Edwards and Jones 2001). In most lizards, elevated plasma estradiol concentrations are associated with vitellogenesis, with a peak in titre during the pre-ovulatory phase (Callard et al. 1992; van Wyk 1994; Jones et al. 1997). Ovarian estradiol switches on a series of events that lead to the production and secretion of vitellogenin (Ho 1991). Plasma progesterone concentration seems to be more important during gestation (Jones and Swain 1996). In general, plasma progesterone titres are low during pre-vitellogenesis and throughout vitellogenesis, then once
1.5 **Extended gestation in lizards from elsewhere in the world**

A well-studied example of extended gestation is the group of skinks in the genus *Niveoscincus*, which are endemic to Australia. This genus has two distinct reproductive strategies occurring in closely related species with overlapping ranges (Wapstra et al. 1999). Lowland to subalpine species such as *N. ocellatus* and *N. metallicus* conform to the conventional patterns of reproductive timing by displaying annual reproduction (James and Shine 1985; Wapstra et al. 1999), with autumn and/or spring mating, ovulation in spring, summer gestation and parturition in late summer (Jones and Swain 1996; Jones et al. 1997). Alpine species occurring at elevations between 800 and 1300 m above sea level, such as *N. greeni* and *N. microlepidotus*, are constrained by severely cold temperatures (Olsson and Shine 1998). In these two species mating occurs in summer, with ovulation the following spring. The females carry developing young *in utero* for approximately one year, giving birth the following spring (although embryos complete development prior to winter) (Olsson and Shine 1998, 1999; Girling et al. 2002). This phenomenon is known as biennial reproduction and is unusual in viviparous lizards. Only a few other known species, including a New Zealand gecko, exhibit such a reproductive strategy (Cree 1994; Olsson and Shine 1999).

1.6 **Lizards of New Zealand**

New Zealand has a large number of endemic reptiles. All but one of the lizard species (and the two tuatara species) are viviparous, and many are unstudied with respect to patterns of reproduction (Cree 1994). However, the majority of those that have been studied show low rates of annual reproductive output and great longevity (Cree 1994; Cree and Guillette 1995; Sheehan 2002). The jewelled gecko *Naultinus gemmeus* exhibits annual reproduction but has an extended gestation of seven months (Wilson and Cree 2003). Limited information on Duvaucel’s gecko *Hoplodactylus duvaucelii* suggests extended gestation and the possibility of
less-than-annual reproduction (Cree 1994). In regard to the common gecko *H. maculatus*, some populations have a five-month gestation period (Turakirae Head, Quail Island and Alexandra), whereas those in cooler areas (Macraes Flat and Middlemarch) carry near full-term embryos over winter, resulting in a 14-month gestation and biennial reproduction (Cree 1994; Cree and Guillette 1995; Rock 1999; Rock and Cree 2003). This biennial cycle is similar to that described above for *Niveoscincus microlepidotus* in Tasmania, Australia (Olsson and Shine 1998, 1999; Girling *et al.* 2002).

Biennial reproduction with extended gestation has not been documented in New Zealand skinks (Cree 1994; Wilson 1998). Two skinks from the same subalpine area as *H. maculatus* at Macraes Flat, the Otago skink (*Oligosoma otagense*) and grand skink (*O. grande*) are inferred to be annual breeders with a gestation length of 4-5 months (Cree 1994). There is only limited data on these two species using non-terminal techniques as they are too threatened for terminal studies. The gestation length of another skink inhabiting the cold subalpine site at Macraes Flat, McCann’s skink (*O. maccanni*), has not yet been determined. Limited information suggests that *O. maccanni* has a gestation of about 4 months, but much of the information is anecdotal, for lower altitude sites and/or confused by subsequent changes in taxonomy that make species identity uncertain (MacAvoy 1976; Patterson and Daugherty 1990; Cree 1994). McCann’s skink is also not classified as threatened under the New Zealand Threat Classification System (Hitchmough 2002), and therefore is more appropriate for terminal studies than threatened species of *Oligosoma*. This thesis therefore focuses on the McCann’s skink population from the subalpine site of Macraes Flat.
1.7 Thermoregulation in Macraes Flat lizard populations

Common geckos (Hoplodactylus maculatus) are primarily nocturnal, but thermoregulate during the daytime by maintaining close contact with the underside of warm rocks (Rock et al. 2000; Rock et al. 2002). Unlike H. maculatus, the skinks O. otagense and O. grande are strongly diurnal and heliothermic (sun-basking) (Cree 1994). It may be that these diurnal skinks can achieve shorter gestation than in common geckos by maintaining higher daytime body temperatures in summer (Wilson 1998). Like O. otagense and O. grande, O. maccanni also achieve high daytime body temperatures at this site (Wilson 1998). In Wilson’s (1998) study it was found that pregnant females of O. maccanni and O. otagense had significantly higher mean body temperatures (adjusted for ambient temperature) than H. maculatus. This same trend, although not significant, was also seen with O. grande (Wilson 1998). It is known that temperature can affect many aspects of development of reptilian embryos. In cool temperate environments, an increase in body temperature often speeds up embryonic development, therefore reducing gestation length (Shine 1980; Beuchat 1988; Wilson 1998). It is therefore suggested that gestation length is influenced by maternal body temperature in the three species of skink and the common gecko mentioned (Wilson 1998).

1.8 Relative clutch mass

The relative amount of effort a female lizard invests into each reproductive episode can be estimated by calculating the relative clutch mass (RCM). This is the total mass of newborn offspring relative to their mother’s body mass. These values can be plotted to compare within species to investigate whether clutch mass changes as maternal mass increases. They can also be plotted to investigate possible differences in investment between different populations of the same species, or between different species.
There is almost nothing known of RCM in New Zealand lizards. Rock (1999) compared RCM of *H. maculatus* from Macraes Flat (biennial reproducers) to *H. maculatus* from Alexandra (annual reproducers) (both undergoing laboratory gestation) and found RCM to be greater in females from Alexandra. In other words, individuals from Alexandra that were the same size as individuals from Macraes Flat, had a significantly larger clutch mass, and this was due to having larger individual neonates (Rock 1999). Wilson and Cree (2003) estimated the RCM for *Naultinus gemmeus* and suggested that this species may produce relatively large offspring (and hence have a relatively large RCM) compared with *H. maculatus* from Macraes Flat. Having relatively large offspring would be an advantage for *N. gemmeus* as young are born in autumn, and being larger would probably increase survival over winter (Wilson and Cree 2003). Another relationship between gestation length and RCM is that a female could invest less into the clutch (by having relatively small individual neonates and/or a relatively small clutch size), thereby reducing gestation length (Tinkle 1969).

### 1.9 Reproduction in female McCann’s skinks

McCann’s skink (*O. maccanni*) is a small (up to 73 mm SVL), diurnal, heliothermic species endemic to the southern half of the South Island (Patterson and Daugherty 1990). It inhabits tussock grasslands and is commonly found under slabs of schist rock and basking on rocks (Patterson and Daugherty 1990). Although some anecdotal information is available on life history of *O. maccanni* (Patterson and Daugherty 1990), the female reproductive cycle of this species has not been studied under subalpine conditions. In this study, I aimed to determine whether female *O. maccanni* show the same unusual pattern of biennial reproduction as female *H. maculatus* do at the same subalpine site (Macraes Flat), or whether they have annual reproduction and are thus more similar (phylogenetically and in terms of thermoregulation) to their relatives, *O. otagense* and *O. grande*. 
I also wished to determine whether female *O. maccanni* invest less in their offspring than *H. maculatus* as a potential trade-off for annual reproduction, if present. In other words, I examined whether they produced a smaller RCM than that reported for *H. maculatus*.

Several techniques can be used when assessing the reproductive condition of a lizard species. In this study, I have used palpation, dissection, and plasma progesterone analysis to estimate and determine reproductive condition and clutch size of female *O. maccanni*. Profiling the plasma sex steroids in viviparous squamates is a valuable tool when studying reproductive cycles. If a sufficient blood sample can be taken without harming the animal, the concentration of sex steroids can indicate the reproductive stage of the female without using dissection. Another useful tool for studying the reproductive cycle of some reptiles is palpation. Palpation involves gently restraining the animal in one hand and applying light pressure to the abdomen using the thumb and first one or two fingers of the other hand (Medica et al. 1971). Palpation is accurate in determining reproductive condition in *H. maculatus* (Cree and Guillette 1995; Girling et al. 1997; Rock 1999), but its use has not been documented for *O. maccanni*. The accuracy of palpation may differ among species and it can be difficult without experience or guidance. I aim to provide guidance on the use and accuracy of this technique in *O. maccanni* and other species. Sexing individuals can be carried out by hemipenial eversion in males by applying pressure and rolling a thumb up the side of the tail towards the cloaca (Wapstra and Swain 2001; Wilson and Cree 2003). Individuals without evertable hemipenes are inferred to be female. Dissection was used to confirm information from palpations and plasma progesterone assays. Although most reliable, dissection is also terminal and therefore cannot be used on threatened species. It is therefore important to test the accuracy of non-terminal techniques that can be applied to threatened species.
The information gathered from this study will assist in understanding the evolution of reproductive patterns among New Zealand lizards, and potentially in conservation management. For instance, it may be that New Zealand skinks consistently have shorter gestation than New Zealand geckos, and this may limit the geckos’ capacity to sustain their populations in the face of environmental pressures. By comparing patterns in different species at the same site, I can test ideas about whether there are fundamental differences between geckos and skinks in length of gestation and whether or not these are related to patterns of RCM and/or thermoregulation.

1.10 Aims, hypotheses and structure of thesis

The main aim of this thesis is to determine whether the reproductive cycle of female *O. maccanni* at Macraes Flat is annual or biennial. I did this by examining the timing of the major reproductive stages (vitellogenesis, ovulation, gestation and parturition), using a combination of techniques: palpation, dissection and hormonal analysis. I hypothesised that *O. maccanni* would be annual breeders, due in part to their phylogenetic relationship with *O. otagense* and *O. grande*, and also to their similar pattern of thermoregulation. *O. maccanni* are diurnal and reach high body temperatures (Wilson 1998), which may allow them to complete embryogenesis more rapidly than common geckos. This aim is covered in Chapter Two of the thesis.

My second major aim is to compare the relative clutch mass (RCM) of *O. maccanni* to existing values for *H. maculatus* from the same area. Providing *O. maccanni* are annual breeders, I hypothesise that they will have a smaller RCM compared to *H. maculatus*, as this may be a trade-off that allows the completion of their reproductive cycle within one year. This
aim is covered in Chapter Three. I also include comparative data available for a diurnal gecko, *Naultinus manukanus*, in this chapter.

The third aim of this thesis is to determine the accuracy of palpation relative to dissection in estimating reproductive condition and clutch size of *O. maccanni*. I hypothesised that palpation would accurately distinguish vitellogenic females from pregnant females. Additionally, I aimed to determine how accurate hemipenal eversion is in determining sex in *O. maccanni*, and describe characteristics to look for when sexing these skinks. These aims are covered in Chapter Four.

Each of these chapters is structured as a stand-alone study with an Introduction, Methods, Results and Discussion. Following these data chapters is a General Discussion where I aim to integrate and discuss my major findings from this study. Finally, a Reference list follows that includes all articles cited in this thesis.
CHAPTER TWO

The Female Reproductive Cycle of *Oligosoma maccanni*

2.1 INTRODUCTION

The timing of reproductive activity and the amount of reproductive effort vary widely in lizards from around the world. It is feasible to understand and test hypotheses about this variation by studying the life history of different species, especially their adaptations to different environments and the factors that may limit reproductive output. Female viviparous lizards from temperate locations in the northern hemisphere tend to be reproductively active during the spring and summer months and remain reproductively quiescent during autumn and winter (Fitch 1970; Duvall *et al.* 1982; Wilson and Cree 2003). Conversely, studies from temperate locations in the southern hemisphere (New Zealand, Tasmania [Australia], South Africa and South America) have shown female viviparous lizards to be reproductively active throughout much of the year (van Wyk 1989; Cree 1994; Ibargüengoytia and Cussac 1996; Jones and Swain 1996; Jones *et al.* 1997). These lizards often have vitellogenesis over autumn-spring, gestation over spring-summer with parturition still occurring in spring or summer or occasionally autumn, and only short periods of gonadal quiescence, if any at all (Rock and Cree 2003; Wilson and Cree 2003).

One well-studied southern hemisphere example is a population of common geckos (*Hoplodactylus maculatus*) from the subalpine area of Macraes Flat (Otago, New Zealand), in
which females biennially reproduce with a gestation of up to 14 months (Cree 1994; Cree and Guillette 1995; Rock 1999; Rock and Cree 2003). In warmer or lower-altitude parts of New Zealand, females of this species are annual breeders with a gestation of 3-5 months (MacAvoy 1976; Cree 1994; Girling et al. 1997). Macraes Flat is an appropriate place to study reptilian reproductive adaptations and the proximal factors that affect them as in addition to H. maculatus, Macraes Flat is also home of up to six species of skinks. The largest (and rarest) of these species are Oligosoma otagense and O. grande; the most widespread are O. maccanni and O. nigriplantare polychroma; the least conspicuous species are O. chloronoton and O. inconspicuum (Towns et al. 2001). Oligosoma maccanni in particular can often be found under rocks also inhabited by H. maculatus.

There is a lack of accurate information on the ecology and reproduction of these Oligosoma species. Some research shows O. otagense and O. grande to be annual breeders at Macraes Flat, with vitellogenesis during autumn-spring, a gestation of 4-5 months and parturition in summer (Cree 1994), which fits with the southern hemisphere pattern; however, no definitive studies have been carried out due to the now-endangered status of these species. There is limited data available on the reproductive cycle of O. maccanni, O. nigriplantare polychroma and O. inconspicuum at various localities in the southern South Island (MacAvoy 1976; Patterson and Daugherty 1990) suggesting similar cycles to that in O. otagense and O. grande (Cree 1994). However much of the information is anecdotal, for lower altitude sites than Macraes Flat and/or confused by subsequent changes in taxonomy that make species identity uncertain.

The main aim of this study is to determine whether female McCann’s skinks (O. maccanni) at Macraes Flat show the same unusual pattern of biennial reproduction as female H. maculatus
Chapter 2: The female reproductive cycle of *Oligosoma maccanni*

do at this subalpine site, or whether they have annual reproduction and are thus more similar to their relatives, *O. otagense* and *O. grande*. This is investigated by determining the timing of the major reproductive stages in females (vitellogenesis, ovulation, gestation and parturition) using palpation, confirmed by dissection of a sub-set of animals. I hypothesise that female *O. maccanni* will have annual reproduction due in part to their phylogenetic relationship with *O. otagense* and *O. grande*, which are thought to be annual breeders (Cree 1994). Additionally, *O. maccanni* like other *Oligosoma* at the same site, are diurnal and can reach high daytime body temperatures (Wilson 1998), which may allow them to complete embryogenesis more rapidly than *H. maculatus*, which are nocturnal.

To use the subset of females that were dissected to their full potential and to heighten our understanding of the biology of viviparous reptiles in New Zealand, several aspects of reproductive and body condition were also investigated throughout the reproductive cycle. This included investigating the seasonality of ovarian development, embryonic development and size of fat bodies. Abdominal fat bodies exhibit a seasonal cycle in many temperate viviparous lizards, with three general patterns recognised (Derickson 1976; MacAvoy 1976). The first cycle involves the utilization of fat bodies for reproduction only (Barwick 1959; Guillette and Sullivan 1985; Castilla and Bauwens 1990; Brana et al. 1992; Ramírez-Pinilla et al. 2002), the second for hibernation only with enough food being obtained during summer to supply nutrients for reproduction (Khalil and Adbdel-Messeih 1962), and the third involves utilization of the fat bodies for both reproduction and hibernation (Avery 1974; Derickson 1976; MacAvoy 1976; Ramírez-Pinilla 1991; Huang 1998). The only data on fat body cycles for a New Zealand skink from the South Island fits the third pattern (*Oligosoma* spp. as *Leiolopisma zelandica* from Alexandra; MacAvoy 1976). I hypothesise that *O. maccanni* will
also have this pattern with decreases in fat body weight during both follicular growth and hibernation.

It has been reported that many of New Zealand’s reptiles have great longevity and low rates of annual reproductive output (number of offspring/female/year) (Cree 1994; Cree and Guillette 1995; Sheehan 2002). The only information on clutch size in *Oligosoma maccanni* (as *Leiolopisma maccanni*) showed a maximum number of six oviducal ova and a mean of three offspring (Patterson and Daugherty 1990); however, these lizards were from various sites. Therefore, the second aim of this study is to investigate clutch size in *O. maccanni*. In particular, I compare clutch size among different reproductive stages to determine whether atresia (death of ovarian follicles) (Méndez-de la Cruz *et al.* 1993) or abortion (or absorption) of ova and/or embryos (Blackburn *et al.* 2003) takes place.

The third aim of this study is to investigate whether plasma progesterone concentrations relate to reproductive stages of *O. maccanni*. If so, profiles for plasma progesterone concentrations in viviparous New Zealand reptiles could be a valuable non-terminal tool for identifying reproductive condition, especially in the larger threatened lizards such as *O. otagense* and *O. grande*. Some preliminary data for *O. otagense* and *O. grande* suggest a peak in plasma progesterone concentration during mid gestation (A. Cree, University of Otago, pers. comm.) so it would be valuable to know if *O. maccanni* have the same pattern. I hypothesise that plasma progesterone titres will be low during pre-vitellogenesis and throughout vitellogenesis, will increase throughout pregnancy, peaking around mid-gestation and then declining prior to parturition (Bourne *et al.* 1986; Callard *et al.* 1992; van Wyk 1994; Jones and Swain 1996; Jones *et al.* 1997).
2.2 MATERIALS AND METHODS

2.2.1 Description of study site and study species

The study site, ‘Cloverdowns Farm’ near Macraes Flat, is a subalpine environment made up of tussock grassland and schist rock outcrops (tors) about 100 km north-west of Dunedin (Fig. 2.1). Collection sites were 600-700 m above sea level. Summer days can be very hot and dry but nights are generally much cooler, and extreme weather such as snow, hail and fog can arrive unexpectedly at any time. Rock et al. (2000) recorded microhabitat temperatures at Macraes Flat by positioning temperature probes under loose slabs. Temperatures reached a maximum of 34°C and a minimum of –1°C during spring/late summer. *Oligosoma maccanni* are a small diurnal lizard species endemic to the southern half of the South Island of New Zealand (Patterson and Daugherty 1990). They are avid sun baskers, commonly found basking on rocks during fine weather where they can reach body temperatures of 33°C (Wilson 1998). The skinks are also often found amongst the vegetation and under loose rock slabs, the latter being common during colder weather when their body temperature can lower to 7.2°C (present study).
2.2.2 Field palpations and collection

At each of five different months of the year, up to 20 adult female *O. maccanni* were captured and palpated to determine when various stages of the reproductive cycle occur. If biennial reproduction is occurring in the population (i.e. if females in two different reproductive conditions are present in the population at the same time) a sample of about this size is sufficient to detect its presence in any given month. Sampling was not carried out in every month of the year including winter as reproductive conditions do not change over winter in other New Zealand lizards studied (Cree 1994). Also, due to the protected status of *O. maccanni* it would be inappropriate to sample every month, especially when reproductive condition changes slowly in New Zealand lizards. Additionally, lizards are hard to find and access during winter as they retreat to deep rock crevices (MacAvoy 1976). The sampling time periods from spring-autumn were as follows: 12 – 26 September, 15 – 24 October, 3 – 5 December 2002, 15 – 25 January and 4 – 11 April 2003. These dates should be appropriate to reveal the times that gestation begins and ends if reproduction is annual as in Otago and grand skinks (Cree 1994).

Skinks were captured by noosing or hand capture, and sexed by examination of the cloacal area for hemipenes. This involved pulling back the cloaca slightly and rolling a finger up the side of the tail, to the base of the tail. Those skinks with hemipenes (inferred males) were released along with the juveniles that were caught. Small lizards approaching adult size were classed as immature if hemipenes and female reproductive structures could not be detected. Females were assessed as to inferred reproductive condition (vitellogenic, early-mid pregnant, late pregnant or non-pregnant) as established for *Hoplodactylus maculatus* by Cree and Guillette (1995), by gently palpating the abdominal cavity between finger and thumb. In *H. maculatus*, vitellogenic follicles are hard spherical structures that roll between fingers like ball
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bearings. Ova during early-mid pregnancy are more ovoid, softer and larger than vitellogenic follicles. Ova during late pregnancy are softer and larger than ova during early-mid pregnancy and the abdomen of the females is distended. The shape and sometimes movement of embryos can be felt or seen through the abdomen of late-pregnant *H. maculatus* (Cree and Guillette 1995). Evidence for the high accuracy of palpation for detecting reproductive condition in *H. maculatus* is summarised by Wilson and Cree (2003).

After field palpation, adult females were weighed to the nearest 0.5 g using a hand-held balance, measured using a ruler (snout-vent length [SVL], vent-tail length [VTL] and length of tail regeneration [R]) and given a temporary pen mark (number written on dorsum with non-toxic pen). Each sampling period seven of the (up to) 20 females were collected for dissection and the remaining females were released back where they were found (except in January, see Chapter 3).

2.2.3 Blood collection

The ideal method of blood collection for the use in hormone assays is to obtain the blood immediately upon capture. This is to reduce the possible effects of elevated corticosterone (Moore *et al.* 1991), which might then affect levels of progesterone. Although holding lizards overnight is not ideal, it is still acceptable because work on some Tasmanian skinks involved holding animals overnight and a clear hormone profile was still obtained (Jones and Swain 1996; Jones *et al.* 1997).

A pilot study was carried out to determine the most appropriate method for collecting blood from adult females. The first attempt involved inserting a heparinised capillary tube into the sub-orbital blood sinus, which can be accessed through the mouth at the angle of the jaw in
some other skinks (Cartledge 2000). The mouth of the lizard can be opened by brushing its lips or gently inserting a fingernail between the lips, both which usually cause the lizard to open its mouth voluntarily (J. Girling pers. comm.). However, this technique was unsuccessful with *O. maccanni* as the lizards would not voluntarily open their mouths and could not safely be forced to open them to allow access to the sub-orbital sinus.

The second method attempted involved collection of blood from the caudal vein. The base of the tail was swabbed with ethanol and a sterile, heparinised needle (25 g) attached to a 1 ml syringe was inserted. This technique has been successful with larger-bodied reptiles including skinks (Edwards and Jones 2001), geckos (Girling and Cree 1995) and juvenile tuatara (Blair et al. 2000). Although feasible in the largest *O. maccanni* without causing harm, it proved very difficult with most specimens to obtain a large enough blood sample over a time period suitable for hormone analysis. Given the very small size of these skinks, the difficulty of the technique, and the need to dissect at least some skinks to verify reliability of palpation and to obtain more detailed, quantitative information on reproductive condition, I decided that blood sampling would be conducted at dissection.

### 2.2.4 Dissections of female *Oligosoma maccanni*

The seven females collected for dissection in each sampling period were placed in cloth bags, transported back to the Department of Zoology and held overnight at room temperature in individual containers (300 x 300 x 150 mm) containing retreat sites made of ceramic tiles for cover, and water dishes. No food was supplied. They were euthanased the following morning by inhalation of halothane vapour followed by cervical transection of the spinal cord. Abdominal palpation was again carried out on each skink after halothane inhalation (except for the September females; see Chapter 4). Blood samples were collected from the severed
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neck in heparinised capillary tubes. Red blood cells and plasma were separated using a centrifuge (12,000 g, 3 min) and stored in separate eppendorf tubes in a -70°C freezer.

The abdomen was then cut open to expose the reproductive organs. The following details were recorded: number of vitellogenic follicles in each ovary, diameters of ovarian follicles, combined mass of ovaries, number and diameter of corpora lutea in each ovary, number of ova in each oviduct, length x width of each oviducal ovum, and combined mass of abdominal fat bodies. Measurements were carried out using a pair of vernier callipers and weights were measured to the nearest 0.01 g on a digital balance. For ethical reasons, embryos of late pregnant females were removed from the oviducts and exposed to halothane vapour before preservation.

Preserved embryos and yolk from the uteri of pregnant females were later weighed. Embryos were dissected from their yolk and all uterine and/or placental membranes removed. Only wet masses of embryos were taken as embryos were used in a separate study of sexual differentiation (Yeong 2003) and consequently could not have been dried before weighing. Yolks were weighed as wet mass then dried in an oven at 60°C to constant mass and weighed again. The embryos were measured (SVL, VTL), and staged collaboratively by C. Yeong and myself according to the scheme developed for *Lacerta vivipara* by Dufaure and Hubert (1961), as reported in Porter (1972).

2.2.5 Progesterone radioimmunoassay

Thawed plasma aliquots (20 μl) were extracted once with freshly redistilled dichloromethane and reconstituted in phosphate buffer. Recovery of tritiated progesterone using this procedure
was 95.4 ± 6.1% (mean ± CV). Plasma progesterone concentrations have been corrected for mean recovery. Plasma samples were analysed in duplicate using a progesterone radioimmunoassay as described by Coddington and Cree (1995) and validated for plasma from *O. maccanni*. Tritiated label was obtained from Amersham, UK, and the antibody (progesterone P11-192) from Endocrine Sciences, California. Cross-reactivities of the antibody were supplied by Endocrine Sciences: 4-pregen-20β-ol-3-one 1.3%, 4-pregnen-20α-ol-3-one 0.8%, deoxycorticosterone 3.3%, and 19 others ≤0.6%. Extracted plasma aliquots and antibody were incubated overnight at 4°C followed by separation of bound and unbound phases by the addition of dextran charcoal followed by centrifugation (4°C, 3500 rpm, 15 min). Radioactivity of the supernatant was counted and curve-fitting performed using a scintillation counter (Wallac 1450 Microbeta, Turku, Finland). The minimum detectable concentration in 20 μl of plasma (after corrections for dilution of plasma during extraction) was 0.54 ng/ml, and all samples had detectable levels. Solvent blanks were consistently non-detectable. The intra-assay CV was 12.2% and inter-assay CV 10.1%. Plasma samples from different reproductive conditions and months were distributed evenly among and within the two assays to minimise effects of any assay variation. A pooled plasma sample with high progesterone showed parallelism with the standard curve when serially diluted. Progesterone added to a pooled plasma sample with initially low progesterone was quantitatively recovered (observed concentration = −0.477 + 0.927 × expected concentration, $r^2 = 0.992$, $F_{1, 3} = 520.55$, $P < 0.001$).

### 2.2.6 Museum specimens

Skink specimens held at the Otago Museum were examined to see if additional information on female reproductive condition for subalpine *O. maccanni* could be obtained. This required
information from each specimen on collection date and location. Although the specimens were useful for learning internal anatomy, most were either male or did not have the required information and so no data are presented.

2.2.7 Statistical analysis

A one-way analysis of covariance (ANCOVA) was used to compare total ovarian mass and diameter of largest ovarian follicles among reproductive groups, with maternal SVL as a covariate. Regression analysis was used to investigate the relationship of maternal SVL on reproductive characteristics. A one-way analysis of variance (ANOVA) was used to compare preserved mass of yolk, preserved mass of embryos and the total mass of the ova (yolk + embryo) (mean ± SE) among pregnant females.

Clutch size can potentially vary between vitellogenesis and pregnancy in two ways. Atresia is a common phenomenon in vitellogenic females of some species of lizards, and refers to the situation in which growing ovarian follicles of all sizes (but more commonly larger ovarian follicles) die and breakdown prior to ovulation (Méndez-de la Cruz et al. 1993). Pregnant viviparous females also have the potential to reduce their clutch size by either spontaneous abortion (after varying periods of retention) or resorption of developing embryos and unfertilised ova (the latter remaining controversial) (Blackburn et al. 2003). Therefore, clutch size was compared among reproductive conditions using ANCOVA with maternal SVL as a covariate and regression analysis was used to investigate the possible relationship of maternal SVL with clutch size.

A one-way ANOVA was also used to compare total fat body mass and plasma progesterone concentrations (mean ± SE) among reproductive groups. Log transformation was used when
variances were heterogeneous. Statistical tests were performed using Minitab 14. If a significant difference was present, posthoc tests (Tukey's) were used to find which groups were significantly different from each other. Significance was accepted at a family error rate of 0.05 (individual error rate of 0.02 when comparing among three groups and 0.007 when comparing among five groups).
2.3 RESULTS

2.3.1 Female reproductive cycle in wild *Oligosoma maccanni* as inferred from palpations

A total of 142 *Oligosoma maccanni* were captured on Cloverdowns Farm between September 2002 and April 2003. Of these, 39 had evertable hemipenes and were therefore inferred as being adult males, so were released immediately. A further 21 individuals were recorded and released as immature on the grounds of small body size (≤ 55 mm SVL) with hemipenes or female reproductive structures not detectable by palpation. The minimum size of maturity in the remaining females was 50 mm SVL (although some individuals were classed as immature up to 56 mm SVL if no reproductive structures could be felt during palpation) and the maximum size of a mature female was 73 mm. Mean SVL ± SE of mature females (n = 77) was 60.83 ± 0.58 mm.

Among the 77 adult females palpated in the field, there was variation among months in reproductive activity (Fig. 2.2). Dissection of a subset of seven females each month confirmed that palpation was accurate in all but two trivial cases, both in the first sampling month of September (early spring). In the full sample of palpated females in September (n = 15), 66.7% were palpated as in late vitellogenic condition, with ovarian follicles estimated as 5-6 mm diameter, and the remaining 33.3% as in early pregnancy (Fig. 2.2). The reproductive condition of five females was correctly determined by palpation when later dissected, but two females palpated as vitellogenic were pregnant when dissected approximately 24 hours later (see Chapter 4). It is unknown whether this reflects inaccuracy in palpation or overnight ovulation. In any case, the effect on the reproductive cycle inferred from figure 2.2 is trivial,
and larger, and hard to distinguish from each other). There was a clear distinction between early and late pregnancy using palpation, but mid-pregnancy was an intermediate stage where it was harder to commit a stage of pregnancy to each female. For a more detailed description of differences between reproductive stages determined by palpation see Chapter 4.

All females palpated in April (early autumn) were in early vitellogenic condition, with vitellogenic follicles approximately 2-4 mm in diameter (Fig. 2.2). Two neonates with placental scars were caught and measured in April and were 27 and 28 mm SVL. Other neonates were sighted during this month but not captured.

2.3.2 Ovarian characteristics throughout the reproductive cycle

Total ovarian mass (combined mass of both ovaries) was heaviest during the late vitellogenic stage in female *Oligosoma maccanni* (Fig. 2.3). When SVL was included as a covariate, ANCOVA revealed that the slopes were heterogeneous when the data were log transformed ($F_{4, 25} = 6.45, P = 0.001$). Regression analysis within reproductive condition showed that SVL had a strong positive influence on total ovarian mass in late vitellogenesis ($F_{1, 3} = 31.21, r^2 = 0.883, P = 0.011$). There was also a positive but smaller influence in early vitellogenesis ($F_{1, 5} = 8.23, r^2 = 0.547, P = 0.035$) and in early pregnancy ($F_{1, 7} = 5.82, r^2 = 0.376, P = 0.047$). Total ovarian mass was not significantly affected by maternal SVL in mid pregnancy or late pregnancy ($P \geq 0.251$).
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Fig. 2.3 Total ovarian mass in relation to maternal SVL of female *Oligosoma maccanni* during different reproductive conditions (each point represents a different female). Maternal SVL only influenced the total ovarian mass in early-vitellogenic, late-vitellogenic and early-pregnant females. Sample sizes are in parentheses in the legend.

The diameter of ovarian follicles did not change following preservation in vitellogenic females ($P = 0.88$). Slopes of the relationships between maternal SVL and diameter of the largest ovarian follicle were significantly heterogeneous ($F_{4, 25} = 3.91$, $P = 0.013$) among reproductive conditions. Log transformation of diameter of the largest ovarian follicle resulted in homogeneity of slopes ($P = 0.113$). When maternal SVL was included as a covariate there was a highly significant difference in diameter of the largest ovarian follicle among reproductive conditions (Fig. 2.4; $F_{4, 29} = 205.02$, $P < 0.001$). Diameter of ovarian follicles was largest in late vitellogenesis (5.42-6.70 mm), followed by early vitellogenesis (2.43-4.37 mm), late pregnancy (1.31-1.68 mm), mid-pregnancy (1.06-1.37 mm), and early pregnancy (0.71-1.40 mm). Within individual reproductive conditions, SVL had a significant influence.
on diameter of the largest ovarian follicle in early vitellogenesis ($F_{1,5} = 9.41, r^2 = 0.584, P = 0.028$) and in late pregnancy ($F_{1,5} = 6.97, r^2 = 0.499, P = 0.046$), but not in other reproductive conditions.

Fig. 2.4 Relationship between log$_e$ of diameter of the largest ovarian follicle and maternal SVL (each point represents a different female). Maternal SVL only influenced ovarian follicle diameter in early vitellogenesis and late pregnancy. Sample sizes are in parentheses in the legend.

2.3.3 Oviducal contents during pregnancy: mass of yolk and embryos

Oviducal ova of pregnant females appeared viable in all but two cases. One female in early pregnancy had two ova, one of which was abnormal in shape and size (constricted in places, thin, of lesser volume, long and irregular in shape). One female in mid pregnancy had two viable ova containing embryos plus one ovum without an embryo. Only ova that appeared viable were included in the following analyses.
Embryos during early pregnancy (October) were < stage 15 and microscopic in size. These could not be dissected out or weighed separately from yolk. Embryos from females in mid-pregnancy (December) were at stage 32 (limb paddles differentiated into the zeugopodium and the stylopodium) whereas embryos from females in late pregnancy (January) were all early-mid stage 40 (scales completely differentiated but still some pigmentation and/or growth to occur). There was a highly significant difference in total wet mass per ovum among all three reproductive conditions ($F_{2, 20} = 119.87, P < 0.001$) (Fig. 2.5). The average total wet mass of ova during late pregnancy was $2.5 \times$ the total mass of ova during early pregnancy (i.e. an increase of 150%). Embryo wet mass significantly increased between mid-pregnancy (stage 32) and late pregnancy (stage 40) ($F_{1, 12} = 146.33, P < 0.001$) (Fig. 2.5). There was a significant difference in yolk wet mass ($F_{2, 20} = 6.45, P = 0.007$), with the difference lying in the decrease of wet yolk mass between mid-pregnancy and late pregnancy (Fig. 2.5). Yolk wet mass was not significantly different between early pregnancy and mid-pregnancy or between early pregnancy and late pregnancy. Maternal SVL did not influence total yolk dry mass of ova during gestation ($P = 0.563$). Yolk dry mass significantly differed among reproductive conditions ($F_{2, 20} = 15.23, P < 0.001$) with a significant decrease between early and late pregnancy and between mid- and late pregnancy but not between early and mid-pregnancy (Fig. 2.5). The average dry yolk mass in ova of late pregnant females (0.0455 g) is 34.55% less than the average dry yolk mass in ova of early pregnant females (0.0695 g). The water content made up 42.3, 57.5, and 57.7% on average of the mass of yolk from ova from females in early, mid- and late pregnancy respectively.
Fig. 2.5 Mean ± SE of total wet mass per ovum (yolk + embryo), embryo wet mass, yolk wet mass and yolk dry mass averaged within female *Oligosoma maccanni* then averaged across females during different stages of gestation. September/October sample = early pregnant females (embryo stages < 15), n = 9; December = mid pregnant females (embryo stage 32), n = 7; January = late pregnant females (embryo stage early-mid 40), n = 7. Yolk wet mass does not significantly differ between early and mid pregnancy or between early and late pregnancy, and yolk dry mass does not significantly differ between early and mid pregnancy. All other relationships are significantly different between reproductive conditions at an individual error rate of 0.02 within each category.

### 2.3.4 Clutch size in dissected females

Clutch size was compared between vitellogenic females and pregnant females in two ways. In both analyses, clutch size in vitellogenic females was the number of vitellogenic follicles per female. Clutch size in pregnant females was calculated firstly as the number of corpora lutea (representing potential clutch size, i.e. the number of follicles that ovulated) and secondly as
the number of viable ova (actual clutch size). These two approaches were taken because in three pregnant females, the number of corpora lutea exceeded the number of viable ova (in the remaining 20 pregnant females, the number of corpora lutea matched the number of viable ova). One female in early pregnancy had two ova but one was abnormal in size and shape as described previously and would not have resulted in a viable offspring; one female in mid-pregnancy had three ova in total but one did not contain an embryo (presumably unfertilised); and one female in late pregnancy had three corpora lutea but only one ovum. For these analyses vitellogenic females were pooled from those in early and late vitellogenesis, as there was no difference in clutch size between females in early vitellogenesis and females in late vitellogenesis (P = 0.093). Similarly, females in early, mid and late pregnancy were pooled as pregnant females as there was no difference in clutch sizes among these groups (potential clutch size: $F_{2, 19} = 32.34$, $P = 0.057$; actual clutch size: $P = 0.140$; although it should be noted that slopes were less homogeneous among pregnant females when using these small sample sizes). This pooling approach was taken to maximise sample sizes and hence statistical power for the comparison between vitellogenic and pregnant females.

Maternal SVL had a significant positive influence on actual clutch size in vitellogenic females ($F_{1, 10} = 39.21$, $r^2 = 0.776$, $P < 0.001$); however, it did not significantly influence actual clutch size in pregnant females ($P = 0.168$). In saying this, it should be noted that there is a positive trend (larger maternal SVL = larger clutch size) for most of the pregnant females, but two of the largest females are outliers. This is also shown when analysing the potential clutch size, as maternal SVL did significantly influence potential clutch size in pregnant females ($F_{1, 21} = 7.57$, $r^2 = 0.23$, $P = 0.012$). Analysis of potential clutch size revealed no difference between vitellogenic and pregnant females ($P = 0.109$) with maternal SVL as a covariate. Mean ± SE potential clutch size for vitellogenic and pregnant females were $3.33 ± 0.26$ and $3.04 ± 0.25$
respectively. Analysis of actual clutch size also showed no significant difference between reproductive groups (Fig. 2.6; \( P = 0.119 \)) when maternal SVL was included as a covariate. Mean ± SE actual clutch size for vitellogenic and pregnant females were 3.33 ± 0.26 and 2.96 ± 0.26 respectively. Ranges in actual clutch size for each of the reproductive conditions are as follows: early vitellogenic = 2-4; late vitellogenic = 3-5; early pregnant = 2-6; mid-pregnant = 2-4; and late pregnant = 1-4.

![Fig. 2.6 Actual clutch size of dissected *Oligosoma maccanni* as recorded from number of vitellogenic follicles for vitellogenic females and from number of viable ova for pregnant females (each point represents a different female and points have been displaced slightly when more than one point exists for display purposes only). Numbers in parentheses can be added to the three affected females to represent potential clutch size (in all other pregnant females, actual clutch size matched potential clutch size; CL = corpora lutea). Maternal SVL significantly influenced clutch size in vitellogenic females only, as represented by the regression line (\( P < 0.001 \)). Sample sizes are in parentheses in the legend.](image_url)
2.3.5 Mass of abdominal fat bodies

Total mass of abdominal fat bodies was compared among the five reproductive conditions (Fig. 2.7). Maternal SVL did not significantly influence total fat body mass \((P = 0.488)\) and was therefore not included as a covariate. Visually, there seemed to be a trend for fat body mass to be heaviest at the start of vitellogenesis (autumn), to have decreased by late vitellogenesis (spring) and to slowly increase throughout pregnancy; however, variation among the samples was not statistically significant \((P = 0.117)\). There were large variances in fat body mass in females in early vitellogenesis and late pregnancy; however, log transformation did not reduce this in females in late pregnancy and there was still no significant difference among samples when log values were used \((P = 0.398)\).

![Graph showing total abdominal fat body mass over the reproductive cycle](image)

Fig. 2.7 Total abdominal fat body mass of female *Oligosoma maccanni* over the reproductive cycle (mean ± SE). Sample sizes are as follows: early vitellogenic = 7; late vitellogenic = 5; early pregnant = 9; mid-pregnant = 7; late pregnant = 7. The variation among reproductive conditions was not significant \((P = 0.117)\).
2.3.6 Plasma progesterone concentration

Variances in plasma progesterone concentrations within reproductive groups were sometimes large so the data were log transformed. Mean log10 plasma progesterone concentrations differed significantly among reproductive conditions (Fig. 2.8; $F_{4, 30} = 26.33$, $P < 0.001$). Females in early vitellogenesis had significantly lower concentrations of plasma progesterone (mean ± SE $1.0 \pm 0.1$ ng/ml) than all other reproductive conditions. Females in mid pregnancy had significantly higher concentrations of plasma progesterone ($15.3 \pm 2.5$ ng/ml) than all other groups. Late vitellogenic, early pregnant and late pregnant females had intermediate concentrations of plasma progesterone and these did not significantly differ from each other. Mean plasma progesterone concentrations in these last three reproductive conditions ranged from $3.5 \pm 0.4$ ng/ml to $5.5 \pm 1.1$ ng/ml.

The average diameter of corpora lutea decreased significantly between early and mid-pregnancy and between mid- and late pregnancy (Fig. 2.8; $F_{2, 15} = 21.13$, $P < 0.001$).
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### Fig. 2.8

Log$_{10}$ plasma progesterone concentrations and diameter of corpora lutea of female *Oligosoma maccanni* in different reproductive conditions (mean ± SE). Lowercase letters above bars indicate significant differences in progesterone concentrations among reproductive conditions ($P \leq 0.007$). Uppercase letters beside data points indicate significant differences in corpus luteum diameter among reproductive conditions ($P \leq 0.02$). Sample sizes are as follows: early vitellogenic = 7; late vitellogenic = 5; early pregnant = 9; mid-pregnant = 7; late pregnant = 7.
2.4 DISCUSSION

2.4.1 Female reproductive cycle in wild *Oligosoma maccanni* as inferred from palpations

If biennial reproduction were occurring in the Macraes Flat population of female *Oligosoma maccanni*, as with *Hoplodactylus maculatus* from the same area, one would expect at the same time to find females in two different reproductive conditions that were not sequential (for example some females would be vitellogenic while others would be in late pregnancy). However, this is not the case with *O. maccanni*. Females instead appear to have a synchronous annual reproductive cycle as inferred through palpation (Fig. 2.2). This pattern, involving autumn-spring vitellogenesis and spring-summer pregnancy, is typical of temperate southern hemisphere lizards (van Wyk 1989; Cree 1994; Ibargüengoytía and Cussac 1996; Jones and Swain 1996; Jones *et al.* 1997).

Vitellogenesis in *O. maccanni* from Macraes Flat appears to span approximately six months, from late February/early March (autumn) through till late September/early October (spring). All females collected during April had early vitellogenic follicles (2-4 mm diameter), so they would have all started vitellogenesis some time after parturition (but possibly before; see below) but prior to April. Although *O. maccanni* were not sampled over winter, due to the large increase in follicle size between April and September, it would not be possible for more than one clutch to be raised during one year. Vitellogenesis lasts slightly longer in *H. maculatus* at Macraes Flat, starting in January and lasting till October (Cree and Guillette 1995; Rock and Cree 2003) (Fig. 2.9).
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Pregnancy seems to last approximately 4-5 months in *O. maccanni*, with ovulation during September/October and parturition in January/February (Fig. 2.9). Although parturition took place between January and April there is reason to believe it took place closer to January/February due to a number of factors. Firstly, embryos from females dissected in January were staged as early-mid stage 40, which is the last number in the developmental scheme. Secondly, five pregnant females held in the lab gave birth between 1-17 February (see Chapter 3). This is consistent with some preliminary data from Macraes Flat (A. Cree pers. comm.) that showed most female McCann’s skinks were still pregnant during January and a few (25%) were still pregnant during February, whereas others were spent. Neonates with placental scars were observed during April in both the present study and by Wilson and Cree (A. Cree pers. comm.). The present data agree with that for plasma concentrations for *O. otagense* and *O. grande*, in which it has also been inferred from palpation and hormone analyses (estradiol, progesterone) that gestation also lasts 4-5 months, with ovulation in October/November and parturition in February/March (Cree 1994). Converse to this, pregnancy in *H. maculatus* lasts approximately 14 months with ovulation during September/October and parturition in October/December, the following summer (Cree and Guillette 1995) (Fig. 2.9).
Fig. 2.9 Comparison of the biennial reproductive cycle of *Hoplodactylus maculatus* (A) (Cree and Guillette 1995; Rock and Cree 2003) and the annual reproductive cycle of *Oligosoma maccanni* (B), both from Macraes Flat. The lighter stippling in vitellogenesis in B expresses the uncertainty about the start of vitellogenesis.

An interesting observation in female *O. maccanni* concerns the possible discrepancies in timing of ovulation between ovaries. Three females palpated in the field during September, but not dissected, were recorded as being late vitellogenic in one ovary but having ovulated from the other ovary. If palpation for these females was correct it is possible that follicles are ovulated from the ovaries at different times, but it is uncertain whether this difference is hours
or days. Indeed, two other females palpated during September were recorded as late vitellogenic but when dissected approximately 24 hours later were revealed to be early pregnant. Assuming the palpations were correct for these females, I hypothesise that ovaries may differ in their timing of ovulation by hours as opposed to days. Palpating obviously late vitellogenic females every few hours could possibly test this.

2.4.2 Ovarian characteristics throughout the reproductive cycle

Ovaries were heaviest and ovarian follicles were largest in female *Oligosoma maccanni* during late vitellogenesis in September (spring). After ovulation, the next clutch of ovarian follicles increased in diameter slightly but steadily throughout gestation, and more rapidly after parturition. The slight increase in size of ovarian follicles over the gestation period is something that does not occur in biennial viviparous skinks such as *Niveoscincus microlepidotus* (Girling *et al.* 2002). It is uncertain as to whether the increase in diameter of McCann's ovarian follicles was pre-vitellogenic or vitellogenic growth. Histology could be used to determine if vitellogenesis was occurring during gestation. Vitellogenic follicles are suspected in some late pregnant *Naultinus gemmeus* from the Otago Peninsula and *H. maculatus* from Alexandra in a laboratory situation, and this may be necessary for completing each reproductive cycle within one year (Rock and Cree 2003; Wilson and Cree 2003).

As the sampling periods for early and late vitellogenesis in *O. maccanni* were separated by winter, it is uncertain whether the growth of vitellogenic follicles occurs gradually throughout this time, or if completion of vitellogenesis ceases temporarily until spring when emergence increases. Deposition of yolk in vitellogenic follicles has been shown to occur over winter in *Oligosoma zelandicum* (Barwick 1959) but sampling would have to take place more frequently over winter and early spring to resolve whether this also happens in *O. maccanni*.
The influence of maternal SVL on total ovarian mass was much greater in late vitellogenic females than it was in early vitellogenic females. Perhaps all females start vitellogenesis at approximately the same time and have an initially similar increase in ovarian mass. As vitellogenesis progresses the larger females might then be able to invest more yolk into their follicles than the smaller females, hence the relationship gets steeper (Fig. 2.3). Similarly, maternal SVL does not influence diameter of the largest follicle in the viviparous skink *Mabuya capensis* (Flemming 1994). Barwick (1959) reported only ‘some’ positive correlation between maternal SVL and ovarian mass, so it would be interesting to see if the late vitellogenic females from Barwick’s (1959) study have a stronger correlation between ovarian mass and maternal SVL if they are separated from early vitellogenic females as the present study shows. Conversely, follicle size is positively correlated with maternal SVL in female *Mabuya mabouya* (Ramírez-Pinilla et al. 2002). Maternal SVL only slightly influenced the total ovarian mass in early-pregnant *O. maccanni* and did not influence it in mid- or late pregnant females. I suspect that this is because the ovaries are more or less inactive during pregnancy, having just ovulated the obviously largest follicles, with only slight increases in ovarian follicle diameters as mentioned above.

### 2.4.3 Oviducal contents during pregnancy: mass of yolk and embryos

There are two pathways by which female viviparous squamates can provide nutritional and water provision to their developing young: vitellogenesis and placentation. There seems to be great variation among species in the degree to which each pathway is utilized (Stewart et al. 1990). Although it seems that most viviparous squamates are lecithotrophic (yolk provides the major source of nutrients for development) based on relative size of the ovulated yolk and neonate, the embryos of many species are dependent on placentae for gas and water exchange (Weekes 1935; Yaron 1985; Stewart 1992; Swain and Jones 1997). In addition to this, it has
been suggested that even relatively lecithotrophic species have incipient placentotrophy that may be used to supplement yolk stores according to nutrient needs (Stewart 1989; Blackburn 2000; Swain and Jones 2000). There are a few species including *Chalcides chalcides*, *Mabuya mabouya*, *Pseudemoia* spp. and possibly *Cordylus cordylus* (van Wyk 1994; Blackburn 2000; Ramírez-Pinilla et al. 2002) in which placentotrophy is the major source of nutrients for embryonic development; yolk mass is generally reduced in these species. It seems that *O. maccanni* is relatively lecithotrophic.

The average total wet mass of *O. maccanni* ova during late pregnancy was 2.5 × the total mass of ova during early pregnancy (i.e. an increase of 150%), with the most significant increase occurring between stages 32-40 of embryonic development. This increase in wet mass during gestation might be caused by maternal-embryo water transfer via the placenta, as has been shown to occur in most other viviparous squamates with varying degrees of placentation. Species showing an increase in wet mass directly after ovulation and another significant increase near the end of gestation include *Mabuya capensis* (Yaron 1985), *Virginia striatula* (Stewart 1989), *Mabuya bristriata* (Vitt and Blackburn 1991), *Sceloporus torquatus* (Guillette and Médez-de la Cruz 1993), *Cordylus giganteus* (van Wyk 1994) and *Cordylus catapharactus* (Flemming and Mouton 2002). In predominantly lecithotrophic squamates the increase in wet mass of the egg can range from 50-200%, most of which represents water obtained from the uterus (Blackburn 1998b).

Growth of the embryos of *O. maccanni* was slow for the first three months of embryonic development and most growth occurred between stages 32-40 as shown by embryo wet mass. Similarly, most embryonic growth occurs between stages 30-40 of embryonic development in *Mabuya capensis* (Flemming 1994). However, if more frequent sampling of *O. maccanni* took
place between stages 32-40 of embryonic development, it might be found that most embryonic growth occurs between stage 40 and parturition, which is true of *Cordylus giganteus* (van Wyk 1994). This would be quite feasible in *O. maccanni* considering the large yolk reserves still present at stage 40.

Although there was a significant increase in the wet mass of *O. maccanni* embryos in the later stages of gestation, this was not paralleled by a concomitant decrease in wet mass of yolk. In fact embryos at a developmental stage of 40 still had no significant difference in wet mass of associated yolk compared to embryos at developmental stages <15. According to the size of stage 40 embryos during January, and information obtained from live females that gave birth in the laboratory during February (see Chapter 3), the stage 40 embryos had only reached approximately 80% of the average SVL and only 53.5% of expected birth mass, and they had about another three or four weeks before birth. Although stage 40 is the last number in the developmental scheme, this stage is prolonged and some authors have used stage 40 to refer to when embryonic development and pigmentation are complete. They then use stage 41 (van Wyk 1994) or stage 40+ (Jones *et al.* 1997) to allow for the growth that occurs from developmental stage 40 to time of parturition. Similar to *O. maccanni*, *Niveoscincus metallicus* embryos reach stage 40 at only 75% of their birth weight, with another three weeks before birth (Swain and Jones 1997).

As *O. maccanni* embryos still had large reserves of associated yolk at stage 40 the question of what happens to the yolk is therefore raised. The yolk associated with *C. giganteus*, *N. ocellatus*, *N. metallicus*, and *N. microlepidotus* embryos is depleted rapidly around late gestation and during the last weeks little or no yolk is associated with the embryos (van Wyk 1994; Jones *et al.* 1997; Swain and Jones 1997; Girling *et al.* 2002). Since *O. maccanni*
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Embryos still had to almost double their expected birth mass before parturition at the time of collection, it is likely that the yolk is depleted in the final stages of gestation in this species also. It is interesting that although the ova from the present study still had a large amount of dry yolk mass in the January (late pregnant) sample (65.5% of that from the early pregnant sample), *Oligosoma* species from Alexandra in December had well developed embryos but no yolk present (MacAvoy 1976). Alexandra (Central Otago, New Zealand) is a lower altitude site and considerably warmer over summer than Macraes Flat (MacAvoy 1976; Cree and Guillette 1995; Rock et al. 2000). Whether or not any yolk is present at the birth of *O. maccanni* from Macraes Flat is unknown. However, there was no evidence from laboratory births (Chapter 3) or literature on other species to suggest that yolk would still be present at the time of parturition.

As dry mass of embryos could not be measured due to samples being needed for another study on sexual differentiation (Yeong 2003), there are uncertainties as to what the relationships between yolk, placentae and embryo are for *O. maccanni*. There remains the question of how the embryo can increase in size when the yolk does not decrease. However, when dry yolk mass is calculated, there is a significant decrease in dry yolk mass throughout gestation. Flemming (1994) found that although embryos of *Mabuya capensis* take up water via the placenta, there is a significant decrease in dry mass of eggs (embryo + yolk) as gestation progresses and late-stage embryos still have large yolk reserves. Flemming (1994) consequently suggested that the yolk supplies most if not all nutritional requirements for this species. This could also be true for *O. maccanni* as, although there is no significant net change in wet mass of yolk, nutrients might still be transferring from yolk into embryo (hence the decrease in dry mass) and water from the placenta could be replacing the nutrients and keeping the overall wet mass of the yolk stable.
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My study does not answer the question as to whether there is additional nutrition supplied to the embryo from the placenta; however, according to the literature, this is quite feasible. Swain and Jones (1997) suggest that although *N. metallicus* is primarily lecithotrophic (Jones and Swain 1996), there is strong evidence for organic uptake via the placenta, which might provide a mechanism for supplementing yolk during gestation in an unpredictable environment. As *O. maccanni* from Macraes Flat has both an unpredictable environment and large yolk reserves, at least to late stages, in common with *N. metallicus*, it could be possible that some organic nutrients may also be supplied to *O. maccanni* embryos via the placenta to supplement the yolk reserves. In order to investigate whether or not nutrients are supplied to *O. maccanni* embryos via the placenta, and to what degree, one could inject radiolabelled nutrients into pregnant females and then assay fetal tissues as carried out on *N. metallicus* (Swain and Jones 1997). Alternatively, the chemical composition of newly ovulated eggs could be compared to neonates as carried out on *Eulamprus quoyii, Elgaria caerulea*, and *Virginia striatula* (Stewart 1992). In addition to finding out about nutrient transport, it would be advantageous to take dry weights of yolk and embryos to help understand embryo development in *O. maccanni*.

2.4.4 Clutch size in dissected females

Clutch size is one of the many reproductive parameters that is subject to geographic and phylogenetic variation. There is evidence that many of New Zealand’s reptiles have low rates of reproductive output (Cree 1994; Cree and Guillette 1995; Sheehan 2002), in part because of low clutch size. Clutch size of vitellogenic females is positively correlated with maternal SVL in *Oligosoma maccanni* and this is true of many other species, for example *Cnemidophorus ocellifer* (Mesquita and Colli 2003), *Sceloporus jarrovi* (Ramírez-Bautista et al. 2002), *Niveoscincus microlepidotus* (Girling et al. 2002), *N. coventryi* and *Pseudemoia*
spenceri (Stewart and Thompson 1998), N. ocellatus (Jones et al. 1997), Takydromus hshuehshanensis (Huang 1998), and Eulamprus tympanum (Schwarzkopf 1992). Actual clutch size of pregnant O. maccanni was not influenced by maternal SVL; however, this was unexpected and is due to two outliers with non-viable ova that reduced their clutch size and pulled the correlation down. The general trend was for larger females to have a larger clutch size and when the analyses were carried out on potential clutch size of pregnant females (as opposed to actual clutch size, therefore removing the outliers) there was a significant positive influence. Maternal SVL does not influence clutch size in H. maculatus as clutch size is fixed in diplodactyline geckos at a maximum of two offspring per reproductive cycle (Cree and Guillette 1995).

Clutch size can vary within lizard populations during different stages of the reproductive season. It is important to know if this occurs in New Zealand lizards in order to avoid overestimation of clutch sizes for conservation purposes and to detect reasons for reproductive failure. Atresia is a phenomenon in which ovarian follicles die prior to ovulation (Méndez-de la Cruz et al. 1993), after ovulation, or at the end of the breeding season (MacAvoy 1976). Some lizard species have very low rates of atresia causing only a small reduction in clutch size such as Niveoscincus ocellatus (0.37%) (Jones et al. 1997), lowland populations of Sceloporus mucronatus (17%) (Méndez-de la Cruz et al. 1993) and N. metallicus (8%) (Jones and Swain 1996). In other species it can substantially decrease the clutch size, for example montane populations of S. mucronatus (54.6%) (Méndez-de la Cruz et al. 1993) and Barisia imbricata (29%) (Guillette and Casas-Andreu 1987). As there was no significant difference in clutch size between early and late vitellogenic female O. maccanni, and between vitellogenic and pregnant females, I suggest that atresia is of minimal significance in this species. These results agree with MacAvoy (1976) who found atretic
follicles to be uncommon in *Oligosoma* species from Alexandra, New Zealand, using histology. Those atretic follicles that were found were present at the start of yolk deposition; with the consequence that nutrients can subsequently be redirected to a smaller number of developing eggs (MacAvoy 1976).

Not only can death of ovarian follicles cause differences in clutch sizes within lizard populations, so too can failure of fertilization or death of the oviducal eggs or embryos. The latter will reduce the clutch size of pregnant females compared to vitellogenic females. Infertile or abortive eggs have been reported in the oviducts of many lizard species (see Blackburn 1998a). They may be retained for varying amounts of time or extruded either as an intact mass or after degeneration to a liquid form, when parturition of viable embryos occurs (Blackburn 1998a; Blackburn *et al.* 2003). Another possibility for the fate of inviable embryos and ova is resorption via the uterus; however, there is no strong evidence that this takes place (Blackburn 1998a; Blackburn *et al.* 2003). One early-pregnant female in the present study had two viable ova and one ovum that was obviously abnormal in size and shape. One mid-pregnant female had two viable ova with visible embryos and another ovum with no embryo present, which was presumably unfertilised. Another female had one ovum with a stage-40 embryo, but three corpora lutea in the ovaries implied that she ovulated three follicles. It is unknown what happens to inviable ova from *O. maccanni* females, but it is feasible that they are retained until near parturition and then expelled. Loss of an ovulated clutch could account for the mature female caught during January that was non-reproductive, but to confirm the presence of corpora lutea dissection would have been required. Although non-viable ova were present in only three females from this study, the sample size was small. Thus, it is uncertain how frequently everts like this occur.
2.4.5 Mass of abdominal fat bodies

Lipids stored as abdominal fat bodies seem to be a critical factor for reproductive success and/or maintenance over winter torpor in lizards living in temperate environments. There was a considerable amount of individual variation in the mass of abdominal fat bodies throughout the different reproductive conditions of female *O. maccanni*. Large variations in mass of abdominal fat bodies were also found in other species such as *Cordylus polyzonus polyzonus* (van Wyk 1989) and *Sceloporus occidentalis* (Goldberg 1974). Although not significant, there was a trend for the fat bodies of *O. maccanni* to be largest at the start of vitellogenesis in early autumn and smallest in late vitellogenesis/early pregnancy during spring/summer. As vitellogenesis coincides with winter torpor it is difficult to discuss the respective roles of abdominal fat bodies without having carried out more frequent sampling over this time period. However, MacAvoy’s (1976) study on *Oligosoma* species at Alexandra shows females have a decrease in abdominal fat bodies during vitellogenesis and “hibernation” whereas female *O. zelandicum* were found to have decreases only during gestation and not winter (which is also when vitellogenesis occurs) (Barwick 1959). MacAvoy (1976) suggested that *O. zelandicum* were able to survive winter without using fat body reserves due to the warmer climate, which enabled them to bask and eat on fine days. Therefore, I suggest that female *O. maccanni* from Macraes Flat are similar to those from Alexandra in that they use abdominal fat bodies for both maintenance during winter torpor and for the reproductive demands of vitellogenesis. I also recommend that fat body mass be investigated in male *O. maccanni* at different times of the year. Males do not have to support vitellogenic growth and therefore it might be easier to discriminate between winter torpor and reproduction in terms of usage of abdominal fat bodies.
2.4.6 Plasma progesterone concentration

Increases in plasma progesterone concentrations in *Oligosoma maccanni* were clearly associated with gestation. Concentrations were lowest during early vitellogenesis, rising significantly prior to ovulation, staying the same until mid-gestation when concentrations peaked and then falling in late pregnancy to similar levels as when late vitellogenic and early pregnant. The major source of progesterone in viviparous squamates is the corpus luteum (Gemmel 1995) so it makes sense that progesterone concentrations are highest during gestation in *O. maccanni* as this is also when corpora lutea are present. I cannot be sure that stress did not cause corticosterone to alter the progesterone levels from time of capture to time of blood collection the following day. However, there was still clear variation in plasma progesterone in a plausible direction. Therefore, I suggest that holding lizards overnight before blood sampling is acceptable in this species as with some Tasmanian skinks (Jones and Swain 1996; Jones *et al.* 1997).

Viviparous squamates exhibit species variation in the morphology of corpora lutea, the timing of luteal regression and plasma progesterone profiles (Jones and Guillette 1982; Jones and Baxter 1991; Callard *et al.* 1992; Martínez-Torres *et al.* 2003). Such that, the major plasma progesterone peak could occur soon after ovulation, in the middle of, or at the end of gestation, depending on the species (Jones and Baxter 1991). Viviparous squamates with low progesterone during vitellogenesis and a peak during mid-gestation similar to *O. maccanni* include the snakes *Natrix sipedon pictiventris* (which has an increase in progesterone when large pre-ovulatory follicles are present, similar to *O. maccanni*) (Chan *et al.* 1973) and *Vipera aspis* (Bonnet *et al.* 2001), and the lizards *Cordylus giganteus* (van Wyk 1994), *Niveoscincus metallicus* (Jones and Swain 1996), *Niveoscincus ocellatus* (Jones *et al.* 1997),
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*Tiliqua* (*Trachydosaurus*) *rugosa* (Bourne *et al.* 1986; Edwards and Jones 2001), *Tiliqua nigrolutea* (Edwards and Jones 2001), *Oligosoma grande* and *Oligosoma otagense* (A. Cree pers. comm.). The biennial female geckos *Hoplodactylus maculatus*, have a plasma progesterone peak directly before and after ovulation and levels are almost basal from mid-pregnancy onwards even though there was approximately 11 months of gestation left (A. Cree pers. comm.).

The specific role of progesterone in gestation in viviparous squamates remains unclear. Progesterone may function to slow the rate of ovarian development by inhibiting pituitary gonadotropins, delaying subsequent ovulations so young are born into optimal conditions (Jones and Baxter 1991; Callard *et al.* 1992; Bonnet *et al.* 2001); it may influence the rate of development of the embryos (Gemmel 1995); it may prepare the uterus by stimulating uterine gland secretions (Jones and Baxter 1991); and/or maintain gravidity by reducing oviducal contractility and maintaining the hypervascularity of uterine tissue (Yaron 1985; Guillette *et al.* 1991; Jones and Baxter 1991; Girling 2002).

It is feasible that some of these ideas on the role of progesterone apply to *O. maccanni*. The significant rise in progesterone between early and late vitellogenesis might function in preparing the uterus for obtaining the ovulated ova. It is possible that plasma progesterone also functions to maintain gravidity in *O. maccanni* as progesterone concentrations decreased prior to parturition. Removing the corpora lutea or the whole ovaries and observing whether or not this causes abortion could test this idea (Callard *et al.* 1972; Jones and Guillette 1982). Alternatively, progesterone could be administered to see if parturition is delayed as in *Sceloporus jarrovi* (Guillette *et al.* 1991). If the idea about elevated progesterone
concentrations delaying ovarian development is correct, the decrease in progesterone in late
gestation might be enough for vitellogenesis to resume before gestation ends (as suspected in
*Naultinus gemmeus* from the Otago Peninsula and *H. maculatus* from Alexandra), which may
be essential in completing each reproductive cycle within one year (Rock and Cree 2003;
Wilson and Cree 2003). As mentioned previously, preserved ovaries of *O. maccanni* could be
used histologically to determine if vitellogenesis begins before parturition.

As a recommendation, progesterone levels should not be based on the size of corpora lutea, as
my study showed that although corpora lutea had significant decreases in size between early
pregnancy and mid-pregnancy, progesterone concentration was significantly higher during the
latter reproductive condition. Therefore, if a decrease in size of corpora lutea is present, it
does not necessarily mean that progesterone concentrations also decrease.

In knowing the pattern of progesterone concentrations in *O. maccanni* not only do we have
more information as a basis for further research into factors that control reproduction in New
Zealand lizards, we also have another valuable non-terminal technique for identifying
reproductive condition in this and possibly other species. Progesterone profiling could be used
in situations where the researcher does not have the appropriate experience or confidence for
using palpation alone as a tool for identifying reproductive condition (see Chapter 4). This is
especially true of larger threatened lizards such as *O. grande* and *O. otagense*, as their size
makes blood sampling feasible from live animals. Additionally, plasma progesterone
concentrations could be used in conjunction with palpation to estimate approximate timing of
events such as parturition, especially in species that are less well studied in terms of
reproduction.
2.4.7 Summary

This study has shown that female *Oligosoma maccanni* from Macraes Flat are annual breeders with a gestation of 4-5 months, as determined using palpation, dissection and plasma progesterone profiles. This is different to female *Hoplodactylus maculatus* from the same site, which are biennial breeders with a gestation of 14 months (Cree and Guillette 1995). This raises the question of how *O. maccanni* can complete each reproductive cycle within one year, while *H. maculatus* takes two years. There are several hypotheses for this variation between these two species and there may be more than one factor contributing to this difference. One hypothesis, which concerns the investment of the mother into the offspring, is that the skinks would have a smaller relative clutch mass (RCM) compared to common geckos. RCM is the total mass of all neonates relative to the mass of their mother. The RCM of *O. maccanni* and *H. maculatus* have therefore been compared and presented in Chapter 3 to investigate whether this is a trade-off that allows annual reproduction in *O. maccanni*. 
CHAPTER THREE

Comparison of Relative Clutch Mass Among Three Species of Lizards

3.1 INTRODUCTION

When studying the life history of a species it is important to investigate what limits a species’ reproductive output. Reproductive output is determined not only by phylogenetic history, but also by natural selection and environmental conditions (Doughty 1996, 1997; Rock 1999). Therefore, there is often more than one variable that determines the reproductive output of a species. One way to investigate some of these variables is to study different species from the same location, especially if there are differences in their reproductive cycles. The female common gecko (*Hoplodactylus maculatus*) from a subalpine environment near Macraes Flat, New Zealand, reproduces biennially, with a gestation of up to 14 months (Cree 1994; Cree and Guillette 1995; Rock 1999; Rock and Cree 2003). However, female McCann’s skinks (*Oligosoma maccani*) from the same area are annual breeders with a gestation of only 4-5 months (Chapter 2). This raises the question of how *O. mccanni* is able to complete each reproductive cycle within one year, whereas *H. maculatus* takes two years at the same subalpine site.
One factor that could explain at least some of the variation in gestation length among lizard species is variation in relative clutch mass (RCM). RCM is the total mass of all neonates relative to the mass of their mother. Interspecific and sometimes intraspecific variation (especially between populations of the same species from different areas) exists in RCM among viviparous lizard species (Doughty 1996; Rock 1999), but little is known about RCM in New Zealand lizards. Rock (1999) compared RCM of *H. maculatus* from Macraes Flat (biennial) to *H. maculatus* from Alexandra (annual) and found RCM to be greater in Alexandra geckos (18% of maternal post-partum mass) than in Macraes Flat geckos (12%) for litters of n = 2. However, these results are from lab-housed females under a warm regime. Gestation in these females was much shorter (approximately 8 months) compared to wild *H. maculatus* from Macraes Flat (up to 14 months; Rock 1999), and it is not known whether the same differences exist in the field-gestated litters. In *Naultinus gemmeus*, a diurnal gecko, RCM was estimated for litters of n = 2 to be 20-24.6% (Wilson and Cree 2003). Embryonic development is completed in about seven months in both *N. gemmeus* and *H. maculatus*, but *N. gemmeus* delivers immediately whereas *H. maculatus* holds full-term embryos over winter and delivers in late spring to early summer (Cree and Guillette 1995; Wilson and Cree 2003). Wilson and Cree (2003) suggested that *N. gemmeus* may produce relatively large offspring (and hence have a relatively large RCM) compared with *H. maculatus*, and this could enhance neonate survival over winter.

In this chapter, I examined variations in RCM among three lizard species. Specifically, I compared RCM between the skink *O. maccanni* and the gecko *H. maculatus* to test the hypothesis that *O. maccanni* will have a smaller RCM compared to *H. maculatus*, as it could be a trade-off that allows completion of the reproductive cycle within one year. I have also
included data available for RCM in *Naultinus manukanus* from Stephens Island (K. Hare pers. comm.), as a representative of this diurnal genus. Gestation length is not known in this species; some females appear to skip some years of reproduction as only 71% of females were pregnant during January 1978 (Hitchmough 1978) and during March 2003 (K. Hare pers. comm.). Assuming that *N. manukanus* has a similar reproductive cycle to *N. gemmeus*, I also hypothesise that *N. manukanus* will have a larger RCM (i.e. relatively large neonates in two-embryo clutches) than in *H. maculatus*.

As diplodactyline geckos have a maximum clutch size of two, females are only able to adjust the size or the quality of their offspring (Doughty 1997). Therefore, I hypothesise, that within each gecko species, larger females will have larger offspring. In contrast, the skink *O. maccanni* has a variable clutch size (maximum of 6; Chapter 2 and J. Marshall pers. comm.), which is influenced by the size of the female, so larger females have relatively more offspring (Chapter 2). Therefore I hypothesise that McCann’s skinks adjust their clutch size and not the size of individual offspring, so that average neonate mass will remain constant and independent of maternal size.
3.2 MATERIALS AND METHODS

3.2.1 Clutch size and mass in lizards

Data for *Oligosoma maccanni* were available from 2003 (present study) and 1999 (J. Marshall pers. comm.). On 25 January 2003, I collected six adult female *O. maccanni* (palpated as in late pregnancy) from Macraes Flat, and transported them in cloth bags to the Department of Zoology. They were held in individual cages (300 x 300 x 150 mm) (L x W x D) containing retreat sites made of ceramic tiles for cover, under a thermal and photoperiodic regime resembling field conditions (natural photoperiod; tile temperature below heat lamp (1000-1600 h) approximately 25°C). They were provided with water *ad libitum* and small locusts (*Locusta migratoria*) and diced pear periodically, and checked daily for births. Neonates were born between 1 - 17 February. When birth occurred, the neonates were counted, weighed and measured (SVL, VTL); post-partum maternal mass and maternal snout-vent length were also recorded. These measurements were added to six measurements of RCM for *O. maccanni* already available to me from another researcher (Jane Marshall, Dept of Botany, OU; pers. comm. to A. Cree) who had births occur during research on feeding preferences in McCann's skinks in 1999. These adult females were collected from Nenthorn (about 8 km SE from Macraes Flat) on 21 January 1999 and young were born from 25 January – 10 February in captivity, held under a similar photothermal regime to that used here (but with greater control over temperatures: night temperature = 13°C; day temperature = 19°C).

Data for *Hoplodactylus maculatus* from the Macraes subalpine site were collected by Rock (1999). Seven of the adult common geckos in late pregnancy were collected on 28 April 1998 and the other eight were collected on 9 October 1998. All these females were palpated as late
pregnant with 2 embryos. Females were held overnight at 15°C (~ 15 hours) and were injected with arginine vasotocin (AVT) the following morning to induce parturition. Females were housed individually at room temperature (21°C) in plastic bins (360 × 360 × 180 mm), with opaque lids and dark paper on the sides. If parturition had not occurred within 24 hours of the first injection, females were anaesthetised with halothane and euthanased by cervical transection of the spinal cord. When birth occurred (or when neonates were dissected from the uteri) neonates and mothers were weighed and measured (Rock 1999, pers. comm.).

Data for *Naultinus manukanus* from Stephens Island (Takapourewa) were collected by Kelly Hare (School of Biological Sciences, Victoria University of Wellington). All adult *N. manukanus* were collected from 9 – 14 March 2003 and births occurred naturally in the laboratory from 25 March – 16 April. Adult *N. manukanus* were housed in groups of three (one pregnant female, one non-pregnant female, one male) in metal enclosures (700 × 580 × 350 mm) with lids that were covered with 1 mm square mesh. Enclosures contained rank pastures grass, leaf litter and tree foliage (*Coprosma repens*) for cover. Room temperature ranged from 16 – 25°C, and photoperiod was on a 12 Light:12 Dark cycle (on at 0600 h). Water and canned pureed pear were provided *ad libitum* and blowflies (*Lucilia sericata*), mealworm larvae (*Tenebrio molitor*) and moths (Lepidoptera) were provided periodically. When births occurred mothers (n = 9) and neonates (n = 2 per litter) were weighed and measured.
3.2.2 Statistical analysis

Clutch size was plotted against maternal SVL for captive births of *O. maccanni* to see if maternal SVL influenced actual clutch size. Clutch mass and average neonate mass per female was calculated to plot against maternal size and compare relative clutch mass (RCM) among species. RCM is the total mass of all neonates relative to the mass of their mother. Individual regression analysis was used to investigate the effect of maternal characteristics on offspring characteristics. One-way analysis of covariance (ANCOVA) was used to compare the relative differences in offspring characteristics among the three species, with maternal post-partum mass or maternal SVL as the covariate. Log transformation was used when samples were heterogeneous. If a significant difference occurred when comparing the three groups, posthoc Tukey’s tests were used to find which groups were significantly different from each other. RCM is not communicated as a percentage in this study as it was in Rock (1999) and Wilson and Cree (2003), as percentages do not take allometric patterns into account. This is important when comparing among species, especially when one species has a fixed clutch size while another has a variable clutch size.
3.3 RESULTS

3.3.1 Information collected from captive births of *Oligosoma maccanni* during 1999 and 2003

Of the six female *Oligosoma maccanni* that I palpated as pregnant in the field in January 2003, five gave birth in the laboratory. The female that did not give birth was recorded as being late pregnant with one embryo when palpated, and had an obviously distended abdomen. This female was often noted basking under the heat lamp set up above her cage in the laboratory, and was reluctant to move from the heat source, similar to late pregnant females in the wild (pers. obs.). Although checked daily, no neonates were found for this female even though she lost a visible amount of weight (from 3.74 g to 3.13 g 14 days later). I suspect that this female gave birth undetected, possibly to a neonate that escaped. This female was left out of all statistics.

For all other female *O. maccanni* from the present study, parturition occurred from 1 – 17 February 2003, an average of 17 days after capture. Female *O. maccanni* from Jane Marshall’s study gave birth from 25 January – 10 February 1999, an average of 11 days after capture. When more than one offspring was born to the same female there were sometimes a time difference of up to 24 hours between the appearance of neonates. It was therefore important to continue thoroughly checking the cage for additional neonates 1-2 days after the birth of the first offspring. The average (± SE) SVL of neonates (first averaged within litter) was 25.2 mm ± 0.5, and the average mass was 0.31 g ± 0.01 (average mass includes data from J. Marshall’s 1999 study). The average (± SE) clutch size for laboratory births (1999 and 2003 combined) was 2.92 ± 0.45, with six offspring as the maximum clutch size. Only two females from the 1999 study had SVL data, therefore only these females could be combined with the
2003 data in comparisons that have maternal SVL as the maternal influence. There was a significant trend for larger female *O. maccanni* to give birth to a larger clutch size ($F_{1,5} = 12.54, r^2 = 0.658, P = 0.017$; Fig. 3.1).

![Fig. 3.1 Clutch size in relation to maternal SVL of female *Oligosoma maccanni* that gave birth in captivity. The number beside the data point indicates there are two data points in that position and they were both females from Jane Marshall’s (unpublished) work. Sample size = 7.](image)

Of the female *H. macculatus* whose data were used in this study, all gave birth within 24 hours of the AVT injection. Clutch size was invariant ($n = 2$ per litter) even though maternal SVL varied (range = 68 – 80 mm). Hence, there is no plot, as maternal SVL does not influence clutch size. All adult *N. manukanus* gave birth from 25 March – 16 April 2003, an average of 18 days since transfer from Stephens Island to the laboratory. As with *H. macculatus*, clutch size was invariant in *N. manukanus* ($n = 2$ per litter) even though maternal SVL varied (range = 71 – 81 mm), hence no plot is needed.
3.3.2 Comparison of relative clutch mass and average neonate mass among three species of New Zealand lizards

The slopes of the relationship between loge clutch mass and maternal post-partum mass for *Oligosoma maccanni*, *Hoplodactylus maculatus* and *Naultinus manukanus* were homogeneous, but only just (F2, 27 = 3.19, P = 0.057). Individual regression analysis showed that post-partum mass significantly influenced the loge clutch mass in *O. maccanni* (F1, 9 = 7.90, r² = 0.408, P = 0.020), but not in *H. maculatus* (P = 0.103) or *N. manukanus* (P = 0.273; Fig. 3.2A). When maternal post-partum mass was included as a covariate there was no significant difference in relative clutch mass among the three species (P = 0.273; Fig. 3.2A). However, it should be noted that there was no overlap of post-partum mass between the skinks and the geckos, making it difficult to compare across families. However, the two gecko species were similar in mass, so I compared these without the skink data. The slopes of the relationship between loge clutch mass and maternal post-partum mass for *H. maculatus* and *N. manukanus* were homogeneous (F1, 18 = 0.00, P = 0.971), and *N. manukanus* had a significantly heavier relative clutch mass than *H. maculatus* (F1, 19 = 15.05, P = 0.001).

When maternal SVL (as opposed to post-partum mass) was included as the covariate for the comparison of total clutch mass among the three species, ANCOVA revealed that the slopes were heterogeneous even once log transformed (F2,23 = 16.62, P < 0.001). Regression analysis within species showed that maternal SVL had a positive influence on the total clutch mass of *O. maccanni* (F1, 5 = 12.35, r² = 0.654, P = 0.017) but did not influence the total clutch mass of *H. maculatus* (P = 0.522) or *N. manukanus* (P = 0.725; Fig. 3.2B). I therefore compared relative clutch mass between the two gecko species only, without the skink data, and found the slopes to be homogeneous (F1, 18 = 0.00, P = 0.956). *N. manukanus* has a significantly
larger RCM than \textit{H. maculatus} when using maternal SVL as the covariate ($F_{1, 19} = 10.57$, $P = 0.004$).
Fig. 3.2 The relationship of loge of total clutch mass as a function of maternal post-partum mass (A) and total clutch mass as a function of maternal SVL (B) for three New Zealand lizard species, *Oligosoma maccanni*, *Hoplodactylus maculatus* and *Naultinus manukanus* (each point represents a different female). Maternal post-partum mass and SVL positively influenced clutch mass of *O. maccanni* only. Sample sizes are in parentheses in the legend.
The slopes of the relationship between average neonate mass and maternal post-partum mass for all three lizard species were homogeneous ($F_{2, 27} = 0.97, P = 0.391$). When post-partum mass was corrected for, there was a significant difference in average neonate mass among all three species ($F_{2, 29} = 22.17, P < 0.001$) (Fig. 3.3A). Maternal post-partum mass did not significantly influence the average neonate mass in *O. maccanni* ($P = 0.403$) or *N. manukanus* ($P = 0.332$). There was a slight trend for heavier *H. maculatus* to have slightly heavier neonates; however, this too was statistically insignificant ($F_{1, 11} = 3.57, r^2 = 0.177, P = 0.085$; Fig. 3.3A). Although regression was insignificant for all three lizard species, visually there is a suggestive weak (but possibly real) effect of maternal post-partum mass on average neonate mass for the two geckos but not for the skinks. Due to this and the lack of overlap in maternal mass with the skinks (Fig. 3.3A), I thought it appropriate to also investigate the gecko data without the skink data. The slopes of the relationship between average neonate mass and maternal post-partum mass for *H. maculatus* and *N. manukanus* were homogeneous ($F_{1, 18} = 0.00, P = 0.969$), and *N. manukanus* had significantly heavier neonates than *H. maculatus* when maternal post-partum mass was corrected for ($F_{1, 19} = 15.79, P = 0.001$).

Among the three species, the slopes of the relationship between average neonate mass and maternal SVL were homogeneous ($F_{2, 23} = 0.03, P = 0.972$). There was a significant difference in average neonate mass between species when maternal SVL was corrected for ($F_{2, 25} = 20.58, P < 0.001$) (Fig. 3.3B). However, maternal SVL did not significantly influence the average neonate mass in *H. maculatus* ($P = 0.522$) or *N. manukanus* ($P = 0.725$) and there was a slightly positive but insignificant trend for *O. maccanni* ($F_{1, 5} = 4.72, r^2 = 0.382, P = 0.082$; Fig. 3.3B). As above, the gecko data were investigated separately to the skink data. Slopes of the relationship between average neonate mass and maternal SVL for the two gecko species...
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were homogeneous \((F_{1, 18} = 0.00, P = 0.956)\), and \(N.\ manukanus\) had significantly heavier neonates than \(H.\ maculatus\) when maternal SVL was corrected for \((F_{1, 19} = 10.57, P = 0.004)\).

![Graph A](image)

![Graph B](image)

Fig. 3.3 The relationship of average neonate mass as a function of maternal post-partum mass (A) and maternal SVL (B) for \(Oligosoma\ maccanni\), \(Hoplodactylus\ maculatus\), and \(Naultinus\ manukanus\) (each point represents a different female). Sample sizes are in parentheses in the legend.
3.4 DISCUSSION

3.4.1 Clutch size of *O. maccanni* from captive births

The mean ± SE actual clutch size of *O. maccanni* calculated from live births from 1999 and 2003 was 2.92 ± 0.45. This is similar to the mean ± SE actual clutch size for vitellogenic females (number of vitellogenic follicles) and pregnant females (number of viable ova), which were 3.33 ± 0.26 and 2.96 ± 0.3 respectively (Chapter 2). When maternal SVL is included as a covariate to compare clutch size among live births, vitellogenic females and pregnant females, there was a slight but significant difference between conditions (F$_{2, 38}$ = 3.22, P = 0.051). This difference is between live births and vitellogenic females, with the latter having the larger clutch size. Of those that gave birth in the laboratory, all were palpated correctly in the field so it is unlikely that this decrease in clutch size was due to captivity. Instead it could be an artefact of a small sample size or due to loss of unfertilised ova.

3.4.2 Influence of maternal characteristics on offspring characteristics

Maternal SVL influences the clutch size of *O. maccanni*: larger females have larger clutches. This is consistent with the results from Chapter 2 in which larger females had more vitellogenic follicles and more embryos (with the exception of two outliers) compared to smaller females. Due to this increase in clutch size with an increase in maternal SVL in female *O. maccanni*, there was also an increase in clutch mass with increasing maternal post-partum mass and SVL. Maternal post-partum mass and/or maternal SVL did not influence average neonate mass per female in *O. maccanni*. This supports my hypothesis that *O. maccanni* adjust their clutch size and not the size of individual offspring, such that larger *O. maccanni* females have larger clutches but the average neonate mass remains constant and
independent of maternal size. It seems therefore that clutch size is determined by the amount of body cavity space and that neonate size is driven by natural selection and/or pelvic constraints (Doughty 1997). I therefore recommend that when examining clutch size in future studies of this species that it be adjusted for maternal post-partum mass or maternal SVL. As larger *O. maccanni* increase their clutch size and not the size of their offspring, it is very unlikely that RCM would have been influenced by the time spent in captivity between collection and birth. This is because at collection all females were in late pregnancy and clutch size at birth matched that palpated at collection.

Surprisingly, maternal post-partum mass and maternal SVL did not significantly influence total clutch mass in *H. maculatus* or *N. manukanus*. Similarly, maternal characteristics of these geckos did not significantly influence average neonate mass per female. However, visually there is a suggestive and possibly real effect of maternal post-partum mass on average neonate mass in both gecko species. Rock and Cree (2003) did find a significant positive influence of maternal post-partum mass on clutch mass and average neonate mass in *H. maculatus* undergoing laboratory gestation. An increase in average neonate mass or the quality of the neonate is the only way in which larger female geckos can increase their clutch mass due to the fixed maximum clutch size of two neonates (Vitt 1986). The regression in the present data may have been stronger if the sample size was larger so it is still important to use a maternal characteristic as a covariate when using average neonate mass in studies on *H. maculatus* or *N. manukanus*. 
3.4.3 Comparison of relative clutch mass and average neonate mass among three species of New Zealand lizards

The main aim of this chapter was to determine if *O. maccanni* have a smaller RCM compared to *H. maculatus*, as this could be a trade-off that allows annual reproduction. Unfortunately, there was not as much overlap in sizes of individuals from the three different species as hoped, making it very hard to compare RCM among species. I therefore cannot make a conclusive statement about whether *O. maccanni* have a smaller RCM compared to *H. maculatus*. The largest female *O. maccanni* was 66 mm SVL and the smallest female *H. maculatus* was 68 mm SVL. There is potential for overlap in SVL between these two species if a larger sample size is taken, because female *O. maccanni* as large as 73 mm SVL were palpated during the course of this study (Chapter 2). Comparisons among species would be more reliable if there were more larger skinks and/or smaller geckos in the analyses. *H. maculatus* from Alexandra are smaller in size compared to those from Macraes Flat (SVL range 55-67 mm and 68-85 mm respectively) and although they are annual breeders like *O. maccanni* there is at least the potential to study some fundamental differences between skinks and geckos. If the relationship was extrapolated out using larger *O. maccanni* or other diurnal, annually reproducing skinks such as *O. grande* (maximum SVL of 106 mm; Cree 1994), it would be possible to test whether annually breeding skinks actually have a higher RCM than *H. maculatus*. If so, there must be other factors that allow female *O. maccanni* to successfully reproduce annually whereas female *H. maculatus* can only reproduce biennially.

One reason that *O. maccanni* may be able to reproduce annually at the same site where female *H. maculatus* reproduce biennially might be to do with the life-history of the species. It is known that lizard species that are long-lived with high survivorship, and delayed sexual
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maturity exhibit low fecundities, whereas those with high fecundities are short-lived, have lower survivorship and early maturity (Tinkle 1969). There is no definitive information on the longevity of *O. maccanni* but as *O. otagense* and *O. grande* are larger-bodied lizards, and live at least 8-9 yr and 4-5 yr respectively (Cree 1994), I suggest that *O. maccanni* probably has a shorter lifespan. There is also no definitive data on the age of maturity of *O. maccanni*. However, it is known that *O. zelandicum* mature at a young age (20-21 months, ~54 mm SVL; Barwick 1959), so owing to the similarities in size and reproduction with *O. maccanni* (ovulation in October, and parturition in January; Barwick 1959) I assume that *O. maccanni* are also early maturing. Converse to this, *H. maculatus* at Macraes Flat are late-maturing, having been estimated to reach sexual maturity at 8 years of age, and have great longevity, living to approximately 31 years of age or more (Sheehan 2002). Thus, it could be that females of *O. maccanni* have a high reproductive effort during each reproductive episode, whereas females of *H. maculatus* have low reproductive effort each reproductive episode, but more of them. So if one was to investigate the life-time reproductive effort of *H. maculatus* from Macraes Flat, reproductive effort might work out to be the same, if not greater, than *O. maccanni*.

An environmental factor that might allow annual reproduction in *O. maccanni* but not in *H. maculatus* is temperature. It is well known that the duration and success of embryonic development in reptiles, which are ectotherms, is temperature-dependent (Cree 1994; Wilson 1998; Rock 1999). As *O. maccanni* are diurnal and *H. maculatus* are nocturnal, the skinks are able to achieve higher daytime body temperatures compared to the geckos (Wilson 1998), which may allow embryonic development to be completed more rapidly. *O. maccanni* are also smaller in body size compared to *H. maculatus*, which may also aid in achieving higher body temperatures at a faster rate (Shine 1980). The energy that a female can invest into her clutch
depends on how much energy is available to her (Pianka and Vitt 2003). There have not been any studies comparing the amount of food available to diurnal and nocturnal lizards at Macraes Flat, but it is possible that there are more or higher energy-containing insects available during the day compared to when *H. maculatus* are active.

It was appropriate to pool data for *H. maculatus* from April and October as embryos are fully developed in April but are held full-term over winter and delivered in October (Cree and Guillette 1995; Wilson and Cree 2003). Additionally, there was no significant difference in mean values of RCM between female *H. maculatus* in April and October (Rock 1999). The diurnal gecko *N. manukanus* has a higher RCM than nocturnal *H. maculatus* from Macraes Flat, and this is driven by having relatively larger neonates than *H. maculatus*. This supports my hypothesis about RCM in diurnal vs. nocturnal geckos. Although little is known about reproduction in *N. manukanus*, no females have been found pregnant over winter (A. Cree pers. comm.) and it is thought that some females have skipped years of reproduction (Hitchmough 1978; K. Hare pers. comm.). Going by data from K. Hare on births in 2003 by captive *N. manukanus*, I speculate that parturition in this species in the wild takes place at a similar time to *N. gemmeus*. The captive *N. manukanus* females gave birth from late March – April (K. Hare pers. comm.) and *N. gemmeus* females give birth in April – June (Wilson and Cree 2003). Parturition in captive *N. manukanus* may have taken place earlier than in the wild if captive temperatures were warmer and more stable than field temperatures.

As with the diurnal skinks, *N. manukanus* might be able to achieve higher daytime body temperatures compared with *H. maculatus*, and thus speed up embryogenesis. Additionally, it is known that there are risks associated with producing and carrying a clutch, some caused by
the physical burden on the female (Shine 1980; Pianka and Vitt 2003). The costs caused by this burden may be greater for _N. manukanus_, which are an arboreal species, compared to _H. maculatus_, which inhabit rock tors, staying in deep crevices over winter. It may therefore be more beneficial for _N. manukanus_ to give birth before winter, and the relatively larger neonates might have a relatively high survival rate over the winter compared to _H. maculatus_.

In conclusion, although RCM of skinks and geckos could not be conclusively compared in this study, there is a suggestion that, relative to maternal size, RCM could be higher in _O. maccanni_ than in _H. maculatus_. This implies there are other factors that are allowing annual breeding in _O. maccanni_, but not in _H. maculatus_ at Macraes Flat. It would be valuable to add more species to this comparison, along with more data for the same species. One species I recommend be included is _Naultinus gemmeus_ as it is a diurnal gecko with a gestation length between _O. maccanni_ and _H. maculatus_; Wilson and Cree (2003) have already made the suggestion that RCM will be greater in _N. gemmeus_ than in _H. maculatus_. Also, _O. grande_ would be useful to include, as they are larger than _O. maccanni_ and overlap in body size with the geckos.
CHAPTER FOUR

Accuracy of Palpation in Determining Reproductive Condition and Clutch Size in female *Oligosoma maccanni*

4.1 INTRODUCTION

Investigation into the reproductive characteristics of reptiles, especially frequency of reproduction and clutch size, is important in understanding evolutionary trends, factors controlling reproduction and to assist in conservation. There are several techniques available to evaluate the reproductive status of reptiles. However, it is important to use an accurate technique for the species in question, while at the same time reducing impact to the population.

Dissection is the most reliable method for determining sex, reproductive condition and potential clutch size in squamates; however, it is also the most extreme. Dissection is best used when the study species is plentiful, but it may also be usefully employed on a sub-sample of the study species to test the accuracy of other methods used (Cree and Guillette 1995; Wapstra and Swain 2001). Another reliable but invasive (though non-terminal) method used for determining reproductive status is laparoscopy. This involves making an incision in the body wall in order to insert an endoscope to examine the reproductive organs (Cree et al. 1990). This method has been used successfully for determining sex and reproductive condition in tuatara and other large-bodied reptiles; however, it is a surgical procedure that
requires training (Cree et al. 1991). Analysis of plasma hormone concentrations can be a reliable way of assessing the reproductive status of female reptiles (Bourne et al. 1986; Callard et al. 1992; van Wyk 1994; Jones and Swain 1996; Jones et al. 1997; Edwards and Jones 2001). Blood samples in larger-bodied species may be drawn from the caudal vein (Girling and Cree 1995; Blair et al. 2000; Edwards and Jones 2001) or the heart (Naulleau and Fleury 1990; Bonnet et al. 2001), or the suborbital blood sinus (Cartledge 2000). However, this technique cannot be used to estimate clutch size, and collection of blood from small lizards may require a terminal method such as decapitation (Bona-Gallo et al. 1980; Jones and Swain 1996). Monitoring of excreted steroids in urine or fecal samples is less invasive but problems with knowing which animal produces the sample, and time delays from the hormones circulating in the blood to being excreted in waste, mean this technique may not be useful in assessing reproductive condition in reptiles (Atkins et al. 2002). Additionally, radioimmunoassay of steroids is expensive and requires time and expertise.

Radiography is another non-invasive technique used to determine reproductive condition and clutch size in reptiles. Cree et al. (1991) X-rayed tuatara to estimate gravidity rate and then used laparoscopy to determine the sex of those that were identified as non-gravid. Clutch size has also been determined in tuatara using radiography (e.g. Newman et al. 1994). In the viviparous lizard *Tiliqua nigrolutea*, radiography was used to identify recently ovulated and late pregnant females (the latter due to calcification of the embryonic skeleton); however, females in early and mid-pregnancy could not be differentiated from non-reproductive females (Gartrell et al. 2002). Radiography is also expensive, and requires an experienced operator. Ultrasound was accurate in determining reproductive condition in *T. nigrolutea*, but was not useful in estimating clutch size in this species or in the freshwater turtle *Chelodina oblonga* (Kuchling 1989; Gartrell et al. 2002). As with laparoscopy and radiography, an
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An experienced operator is needed when using ultrasound, and it is most feasible with large-bodied animals.

Palpation is another technique used to determine reproductive status of female reptiles (Medica et al. 1971; Cree 1994; Cree and Guillette 1995; Girling et al. 1997; Cartledge 2000; Wapstra and Swain 2001; Gartrell et al. 2002; Rock and Cree 2003; Wilson and Cree 2003). Palpation has the advantages of being non-invasive and not requiring any technical equipment, so it can be carried out in the field. It involves gently restraining the animal in one hand and applying light pressure to the abdomen using the thumb and first one or two fingers of the other hand (Medica et al. 1971). Palpation is usually combined with another method on a subset of the sampling population to test the accuracy, especially if it is being used on a new species or population. Wapstra and Swain (2001) found palpation to be 100% accurate in determining clutch size for a coastal population of *Niveoscincus ocellatus*, but unreliable for the same species from a higher altitude, in which clutch size was larger. Palpation is accurate in determining reproductive condition and clutch size in the common gecko *Hoplodactylus maculatus* (Cree and Guillette 1995; Girling et al. 1997; Rock 1999). In this species, vitellogenic follicles are hard and spherical, ova in early-mid pregnancy are ovoid and softer, and in late pregnancy the form of the embryo can be seen and movements may be felt (Cree and Guillette 1995). Accuracy of palpation can be determined by dissection and by documenting live births (Cree and Guillette 1995; Girling et al. 1997). However, although palpation is a widely used technique, apart from the studies of Cree and Guillette (1995) and another on the large-bodied skink *Tiliqua nigrolutea* (Gartrell et al. 2002), there is little information available on what is felt during palpation, or rigorous evidence to support its reliability and document its limitations. This makes it very difficult for anyone wanting to use this technique in other species if they have little or no experience.
Sexing of adult lizards is generally not considered problematic, since male intromittent organs (hemipenes) can be everted, or the hemipenial sac that houses them can be seen (Wapstra and Swain 2001; Wilson and Cree 2003). However, false negative results in some species (e.g. monitor lizards, *Varanus* spp. and the snake *Bothrops insularis*), can occur if females have hemiclitoris that can be everted like male hemipenes (Beçak *et al.* 1990; Böhme 1995), in which case females might be mistaken for males. Although hemipenial eversion has been used to help sex neonatal *Oligosoma suteri* (Hare *et al.* 2002) some results were problematic. The reliability of this procedure in sexing adult New Zealand skinks has not been reported.

As many of New Zealand’s lizards are threatened (Daugherty *et al.* 1994; Hitchmough 2002), it is never going to be possible to get information on female reproduction from terminal techniques. Therefore, it is important to know how reliable palpation is, both for evolution and life-history studies, and for conservation. It is also important to have information on the reliability and procedure of hemipenial eversion for sexing New Zealand lizards.

The main aim of this chapter is to determine the accuracy of palpation in estimating reproductive condition and clutch size of a relatively common New Zealand skink, *Oligosoma maccanni*. Palpation was carried out in live females (unanaesthetised) and again once euthanased, and compared with the results from subsequent dissection. I hypothesised that palpation would allow me to accurately distinguish females in vitellogenesis from those in pregnancy. I also hypothesised that palpation would be less accurate in estimating clutch size in *O. maccanni* with a large clutch size compared to *O. maccanni* with only one or two ova. I also aimed to determine whether absence of hemipenial eversion is accurate in determining sex of female *O. maccanni*. Also, gross female reproductive anatomy has never been
Chapter 4: Accuracy of palpation in female *O. maccanni*

described or illustrated in detail for any New Zealand skinks, so I aim to provide this for female *O. maccanni*.
4.2 MATERIALS AND METHODS

Thirty-five adult female *Oligosoma maccanni* (7 per month in 5 different months) were collected from Cloverdowns Farm near Macraes Flat (Chapter 2). Putative females were selected on the basis of absence of hemipenial eversion. The abdomen was palpated in the field initially and then again in the laboratory after euthanasia one day after capture. Reproductive condition was classified as vitellogenic, early-mid pregnant, late-pregnant or non-pregnant by palpation as established for *Hoplodactylus maculatus* by Cree and Guillette (1995). Clutch size was also estimated by palpation and then determined by dissection. At dissection, the following details were recorded: number of vitellogenic follicles in each ovary to compare dissection with palpations; diameters of vitellogenic follicles to compare the average size between early- and late-vitellogenic females; number of ova in each oviduct to compare dissection with palpations; and length and width of each oviducal ovum to compare average size among pregnant females. Measurements were carried out using a pair of vernier callipers and weights were measured to the nearest 0.01 g on a digital balance. Embryos were dissected from the ova and staged according to the scheme developed for *Lacerta vivipara* (Dufaure and Hubert 1961; Porter 1972). Photographs were taken to illustrate the external differences between male and female *O. maccanni*. The accuracy of hemipenial eversion was assessed in terms of eliminating putative males (and hence, identifying females), not in confirming maleness. Photographs of some dissected females were also taken to provide an illustrated guide on the gross reproductive anatomy of female *O. maccanni* at different reproductive stages. Refer to Chapter 2 for more details on animal collection, palpation, and dissection.
To calculate the mean (± SE) diameter of vitellogenic follicles and ovarian ova, taken at dissection, measurements were first averaged for each female and then averaged across females in each reproductive condition. Repeated measure ANOVAs were used to compare accuracy of palpation for estimating clutch size in the field and after anaesthesia compared with dissection. Clutch size was analysed within reproductive condition to determine when palpation was most accurate in identifying clutch size. If clutch size was uncertain (e.g. 4 or 5) during palpation, the highest estimated value was used in analyses. Statistics were carried out using SPSS 11, with significance accepted at $P < 0.05$. Least Significant Difference (LSD) pairwise comparisons were used to identify where significant differences lay.
4.3.2 Accuracy of abdominal palpation in determining reproductive condition of female McCann’s skinks

Of 35 females palpated, 33 (93%) were accurately identified as the correct reproductive condition, based on dissection the next day (Table 4.1). The two females in which the results differed were palpated in the field during September as vitellogenic, but at dissection the following day they were early pregnant. Palpation was 100% accurate when individuals were palpated in the laboratory after terminal anaesthesia; however, this was not carried out during September.

Table 4.1 Reproductive condition of female Oligosoma maccanni as inferred through palpation in the field, and then determined through dissection, during five months of the year. Sample sizes are in parentheses.

<table>
<thead>
<tr>
<th>Month</th>
<th>Inferred reproductive condition (palpation)</th>
<th>Determined reproductive condition (dissection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April (n = 7)</td>
<td>Early vitellogenesis (n = 7)</td>
<td>Early vitellogenesis (n = 7)</td>
</tr>
<tr>
<td>September (n = 7)</td>
<td>Late vitellogenesis (n = 7)</td>
<td>Late vitellogenesis (n = 5) Early pregnancy (n = 2)</td>
</tr>
<tr>
<td>October (n = 7)</td>
<td>Early pregnancy (n = 7)</td>
<td>Early pregnancy (n = 7)</td>
</tr>
<tr>
<td>December (n = 7)</td>
<td>Mid pregnancy (n = 7)</td>
<td>Mid pregnancy (n = 7)</td>
</tr>
<tr>
<td>January (n = 7)</td>
<td>Late pregnancy (n = 7)</td>
<td>Late pregnancy (n = 7)</td>
</tr>
</tbody>
</table>
Vitellogenic females felt quite empty in the abdominal area (except for the ovaries) so that the abdomen could be pushed flat against the dorsal surface. Ovaries were located approximately half-way between the front and back legs and were quite medially positioned, although they were often displaced during palpation. At the start of vitellogenesis in April, vitellogenic follicles felt small, hard and spherical, like small ball-bearings (Fig. 4.2A). However, with a mean diameter at dissection of only 3 mm (± 0.3), they were easy to miss during palpation, especially when food was also present. The follicles from late vitellogenic females captured and dissected during October were larger and easier to locate than in April. These follicles had a mean diameter during dissection of 6 mm (± 0.2), and felt hard and obviously spherical. Again the ovaries were often displaced during palpation, and follicles were clustered together three-dimensionally in the ovary.

Females in early pregnancy were easily distinguished from vitellogenic females during palpation. Once females had ovulated, uterine ova felt obviously spherical, with a mean length of 7.7 mm (± 0.3) and a mean width of 5.5 mm (± 0.2) (averaged for each female then across females, n = 7). Uterine ova of females in early-pregnancy were positioned quite laterally and could sometimes be seen from the external ventral surface of the live female if she was relaxed. There was a distinct space between the left and right oviduct, and within each oviduct ova were lined up end to end, separated by interembryonic regions that were constricted, forming incubation chambers between them (see Fig. 4.2B). Dissection of females in early pregnancy showed consistently yellow ova, and no embryos were visible to the naked eye; however, staging using a microscope showed most ova to have embryos at a developmental stage < 15.
Ova from females in mid-pregnancy were more medially positioned and softer, with a less ovoid shape than in females in early-pregnancy. The interembryonic regions were still present, but not as distinct. Ova were larger in females in mid-pregnancy, with a mean length of 9.1 mm (± 0.2) and a mean width of 8.0 mm (± 0.4) (averaged for each female then across females, n = 7). The medial positioning and increase in size made it harder to determine whether ova were in the left or right oviduct (Fig. 4.2C). Embryos (stage 32) were positioned on the dorsal surface of the ova.

Females in late pregnancy often had a distended abdomen, which made them look obviously pregnant. They felt more full, with no space for ova to move, and the ova felt larger and much softer than in other females, making it hard to identify where each ovum started and ended, and in which oviduct the ova were located (Fig. 4.2D). Interembryonic regions were no longer present. Ova of late pregnant females had a mean length of 11.7 mm (± 0.5) and a mean width of 10 mm (± 0.3) (averaged for each female then across females, n = 7). When dissected, ova from females in late-pregnancy were not ovoid like those of females in early- or mid-pregnancy. Instead, ova took on a more deformed shape due to the limited space in the abdominal cavity of late-pregnant females. Embryos were at early-mid stage 40, but there was still some pigmentation and growth to occur (see Yeong 2003 for pictures). There was no damage to the females or to their ova or embryos as a result of palpation.
4.3.3 Accuracy of palpation in determining clutch size in McCann’s skinks

It was difficult to determine clutch size in females in early vitellogenesis, as the follicles were small and the ovaries easily displaced. Cold weather added to the difficulty and, to avoid handling animals for an unnecessary amount of time, clutch size was not estimated in the field. I also found palpating for clutch size after anaesthesia to be difficult. Although there was no significant difference in mean clutch size estimated by palpation after anaesthesia with that at dissection for early vitellogenic females ($F_{1, 5} < 0.001, P = 1.0$), clutch size was underestimated by one in two females and overestimated by one in another two females (Table 4.2). Vitellogenic follicles were larger in September than in April, but it was still hard to estimate clutch size. This was due to the displacement of the ovaries during plaption and the three-dimensional shape of the ovaries, which made it hard to determine individual follicles. There was a significant difference in mean clutch size between palpation in the field and at dissection ($F_{1, 6} = 10.8, P = 0.017$) and this was due to underestimating clutch size by one or two follicles for five of the seven females in September (Table 4.2). As September was the first sample, I did not palpate after anesthesia and only began to do so with subsequent samples. The two females that were palpated as in late-vitellogenesis in September, but that were in early pregnancy at time of dissection, have been included in the late-vitellogenic sample for clutch size estimates.

For all samples of pregnant females, clutch size was estimated by palpation in the field, palpation in the laboratory after anaesthesia, and at dissection. Clutch size was much easier to determine in early pregnancy than in vitellogenesis. The single-file positioning of ova, and the oviducal spacing in females in early pregnancy (see Fig. 4.2B) helped considerably in determining clutch size. There was no significant difference in clutch size among the three methods ($F_{1, 6} = 1.0, P = 0.356$). Clutch size was incorrectly estimated by palpation in only...
one female in early pregnancy (a large clutch size of six ova underestimated by one ovum) (Table 4.2). Although ova from females in mid-pregnancy were softer, they were still firm enough to distinguish from each other, and oviducal spacing also helped with estimation. Clutch size in one female in mid-pregnancy palpated in the field was overestimated by one follicle; however, after anaesthesia clutch size was estimated correctly for all these females. Hence there was no significant difference in mean clutch size among methods for females in mid-pregnancy ($F_{1,6} = 1.0$, $P = 0.356$; Table 4.2). However, there was a significant difference in clutch size among methods in late-pregnant females ($F_{1,6} = 15.0$, $P = 0.008$) and this was due to inaccuracy in the field only. In late pregnancy the ova filled the entire abdominal cavity and there was no distinction between left and right oviducts, and no definition of separate ova (see Fig. 4.2D). This caused an overestimation of clutch size by one ovum for most females (Table 4.2). Palpation after anaesthetic was more accurate in estimating clutch size in late-pregnant females, with an overestimation by one follicle in only the first female.
Table 4.2 Mean (± SE) clutch size for *Oligosoma maccanni* using three methods: palpation in the field, palpation after anaesthetic, and at dissection. Sample sizes are in parentheses.

<table>
<thead>
<tr>
<th>Month and palpated reproductive condition</th>
<th>Clutch size estimated by palpation or determined by dissection</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Field palpation</em></td>
<td><em>Euthanased palpation</em></td>
</tr>
<tr>
<td>April (n = 6) (Early vitellogenesis)</td>
<td>-</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>September (n = 7) (Late vitellogenesis)</td>
<td>2.6 ± 0.3*</td>
<td>-</td>
</tr>
<tr>
<td>October (n = 7) (Early pregnancy)</td>
<td>3.4 ± 0.4</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>December (n = 7) (Mid pregnancy)</td>
<td>3.6 ± 0.4</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>January (n = 7) (Late pregnancy)</td>
<td>3.0 ± 0.3*</td>
<td>2.4 ± 0.5</td>
</tr>
</tbody>
</table>

* significant difference in clutch size between field palpation and dissection (*P* ≤ 0.05).

† two females in September were pregnant at dissection but are included as in late vitellogenesis as that was the palpated condition.
4.4 DISCUSSION

4.4.1 Accuracy of hemipenial eversion in eliminating male McCann’s skinks

Eversion of hemipenes appears reliable as a method of sexing mature *O. maccanni*, at least in terms of eliminating males. Converse to this, in MacAvoy’s (1976) study on *O. maccanni*, it was stated that dissection was the only reliable method of sexing this species. In my study, the fact that it was harder to ever hemipenes in individuals that looked male (judged by bulges at the tail-base) during September could be due to inexperience, as this was the first sample, or it could be a seasonal effect (i.e. non-breeding). The testes weight ratio of *Oligosoma* spp. (as *Leiолopisma zelandica*) from Alexandra was at a minimum in September (MacAvoy 1976), therefore it is quite feasible that hemipenes are harder to ever during this time. Hemipenes do not need to be fully eveted to identify a male, and as a precaution, full eversion should be avoided to prevent prolapsed hemipenes (although this did not occur during this study). There are a number of characteristics to help identify males, including one bulge either side of the midline of the tail (where the inverted hemipenes are housed) and enlarged blood vessels inside the cloacal vent. However, eversion of hemipenes is not accurate in sexing neonatal *O. maccanni*, in part because structures are difficult to ever, but also because neonatal females still have equivalent structures (Yeong 2003). Minimum age at which regression of these structures (hemiclitori) occurs in females is unknown (Yeong 2003).
4.4.2 Accuracy of palpation in determining reproductive condition of female McCann’s skinks

Apart from the two females from the first sample (September), palpation was accurate in determining reproductive condition of female *O. maccanni*. The possible inaccuracy in two females in September could be due to inexperience (it was the first sample), or to the females possibly ovulating overnight. Some other females palpated but not dissected during September were recorded as being late vitellogenic in one ovary but having ovulated from the other ovary (Chapter 2). Vitellogenic females were identified as having small, hard, spherical follicles positioned approximately midway down the body, clustered in the highly motile ovaries. These characteristics are similar for vitellogenic *T. nigrohutea* (Gartrell et al. 2002) and *H. maculatus* (Cree and Guillette 1995), except that *H. maculatus* do not have a cluster as there is only one vitellogenic follicle per ovary. Caution is needed when food is present in the gut as this has the potential to cause misdiagnosis (Gartrell et al. 2002).

Females in early pregnancy were clearly distinguishable from vitellogenic females due to the ova being lined up in the oviduct. I will consider females in early- and mid-pregnancy together as apart from a small increase in size of the ova, there was not much difference between these two conditions. Cree and Guillette (1995) also grouped early- and mid-pregnant female *H. maculatus* together. However, *T. nigrohutea* in mid-pregnancy are indistinguishable from non-reproductive females (Gartrell et al. 2002). Ova in early-mid-pregnant female *O. maccanni* felt obviously different from those females in late pregnancy. Early-pregnant females had firm, distinctly ovoid ova that had interembryonic regions, whereas ova in late-pregnant females were much softer and larger so they took up the whole abdominal cavity, causing the abdomen to be distended and taut, and separate structures were unidentifiable. No movements of embryos could be felt or seen in late-pregnant *O. maccanni,*
unlike those observed for *H. maculatus* (Cree and Guillette 1995). Gartrell *et al.* (2002) pointed out the possible risk of follicle or yolk rupture during palpation; however, there was no evidence of this during the present study.

### 4.4.3 Accuracy of palpation in determining clutch size in McCann’s skinks

Palpation is most accurate in estimating clutch size in *O. maccanni* during early-mid pregnancy. This is due to the ova being arranged in longitudinal rows, in separate incubation chambers, with interembryonic spacing. Blackburn (1998b) suggested that interembryonic constrictions in squamates may be due to active or tonic contraction of the uterine musculature, or the chambers may just be sites of oviducal distension. Palpation was inaccurate in determining clutch size in vitellogenic females due to the clustering of follicles in the movable ovary. Although there was no significant difference in clutch size estimated at palpation and confirmed at dissection for early vitellogenic females, only two females were estimated correctly (i.e. there was an equal degree of over- and underestimation). In late pregnancy, ova fill the entire abdominal cavity and separate ova cannot be distinguished. Palpation in the field is not accurate in estimating clutch size in late-pregnant females. Palpation was accurate after anaesthesia in late-pregnant females. I believe this increase in accuracy resulted from observation of the first dissection of the females in late pregnancy, as I then had an idea of what to feel for in subsequent laboratory palpations.

### 4.4.4 Applications of the information obtained on palpation in *O. maccanni*

Many New Zealand lizards are threatened; therefore it would never be possible to get information on female reproductive cycles using terminal techniques. It is important to know how accurate palpation is and to have information on hemipenal eversion for sexing lizards, both for evolution and life history studies, but also for conservation. I suggest that palpation is
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A technique that could be applied to other New Zealand lizard species to accurately distinguish between vitellogenic, pregnant, and non-reproductive females, and to estimate clutch size. Specifically, I suggest palpation will be accurate in lizards similar in size to *O. maccanni*. Palpation could also be extended to larger New Zealand lizard species, however, I cannot confidently say how accurate palpation would be in larger, more robust species. In saying this, as long as another non-terminal technique is applied to confirm palpation, I think palpation should be accurate in most New Zealand lizards.

The information obtained from this study will be most useful for researchers working on threatened species that cannot be dissected. For determining reproductive condition in such species, the use of the information in the present study could be paired with plasma progesterone analyses to increase accuracy. There are several reasons why it is important to determine reproductive condition of female lizards. Firstly, the knowledge increases our understanding of evolution and life-history. Secondly, it is important to know the reproductive cycle in terms of management and conservation. Additionally, it is important to determine if some mature female lizards are not reproducing and to then find out why.

For estimating clutch size most accurately, I recommend palpating females in early-mid pregnancy. If a subset cannot be dissected to test accuracy, I suggest using another method such as ultrasound. There are several scenarios where it is important to have accurate estimates of clutch size in a lizard species. Knowing clutch size of individuals is important in studies on temperature-dependent sex determination (TSD) in viviparous lizards, so differential sex-specific mortality of embryos can be ruled out if clutch size from palpations and births is the same (Wapstra *et al.* 2004). Secondly, in order to conserve a threatened species, it is necessary to understand the reproductive output of the species and to study
changes in the size of the population. There are many different population models available to estimate the size of a population. Some models such as population viability analysis (PVA) are used to predict consequences of different management options, such as predator control, and captive rearing, to identify the most effective management plan (Shaffer 1990). Most population models need several parameters to do with the birth, death, and movement of a population, including clutch size of the species, and sex ratios. In relation to previous studies that have used palpation as a technique to estimating clutch size, but have not confirmed their estimates through dissection, I suggest accuracy will depend on when during the reproductive cycle palpations occurred. Cree (1994) reported the mean clutch size estimated by palpation for *O. grande* to be 2.4 ± 0.1 and for *O. otagense* to be 2.6 ± 0.1. Although these estimates do not seem too far from what I would expect, I suggest that they are not as accurate as they could be. This is because the estimates were taken throughout vitellogenesis (an inaccurate time for *O. maccanni*), and throughout pregnancy, including late pregnancy (also an inaccurate time in *O. maccanni*). Like *O. maccanni*, *O. grande* and *O. otagense* are very difficult to palpate in late pregnancy, as ova are hard to distinguish from each other (A. Cree pers. comm.).

Knowing that palpation is accurate in *O. maccanni* and possibly other New Zealand lizard species is valuable for captive populations. Monitoring the reproductive status of captive populations is useful for adjusting housing conditions and food availability to an individual’s needs. Additionally, studies can be carried out to compare clutch size in captive populations with wild populations, using palpation. If there is a difference, limitations for the wild population could be pointed out and conservation can then be focused on increasing the limiting factor/s.
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The illustrations of gross reproductive anatomy can be applied to museum specimens to compare, for example, the size of reproductive structures relative to the specimen, to help determine reproductive condition. The illustrations would also be helpful to a researcher that had no experience in palpation and was not familiar with the gross reproductive anatomy of a lizard at different times of the reproductive cycle.

In conclusion, although palpation does require some experience and training, it does not take long to learn, especially when descriptions such as the present study are available. Advantages of palpation include the ease of use in the field and the low impact to the animal. Palpation is accurate in identifying vitellogenic, early-mid pregnant and late pregnant female *O. maccanni* and was most accurate in estimating clutch size in early-mid pregnant females.
CHAPTER FIVE

General Discussion

The overall aim of this thesis was to investigate the female reproductive cycle of a diurnal viviparous skink (*Oligosoma maccanni*) from a subalpine site. This included determining the timing of the major reproductive stages and investigating several aspects of reproductive and body condition throughout the reproductive cycle (Chapter 2). Palpation was used to gather the information non-invasively and dissection was used on a subsample to confirm information from palpation. I then compared some of the findings to existing data for a nocturnal gecko (*Hoplodactylus maculatus*) from the same site, and a diurnal gecko (*Naultinus manukanus*) from Stephens Island, in order to investigate differences in relative clutch mass (Chapter 3). The accuracy of palpation in estimating reproductive condition and clutch size in *O. maccanni* was then assessed (Chapter 4). Additionally, a comprehensive description of gross anatomy during each reproductive stage using palpation and dissection was provided.

There is little information available on reproduction in many of New Zealand’s lizards, but what studies have been done show low rates of reproductive output in females and great longevity (Cree 1994; Cree and Guillette 1995; Sheehan 2002). As many of New Zealand’s lizards are threatened (Daugherty *et al.* 1994; Hitchmough 2002), it will never be feasible to get information on female reproductive cycles in all species using terminal techniques. As *O. maccanni* is not threatened, it is an appropriate study species on which to test procedures. The findings presented in this thesis have increased our knowledge of reproductive cycles in New
Zealand lizards, and confirmed procedures suitable for studying them. This information can
be applied to understanding life history evolution, and more importantly to conservation of *O.
maccanii* and other New Zealand lizard species. The following section discusses the major
findings from this research and makes recommendations for future research.

5.1 Reproduction in female *Oligosoma maccanii* at a subalpine site

The main aim of this research was to investigate the timing of reproductive events and several
aspects of reproductive and body condition throughout reproduction of female *O. maccanii* at
Macraes Flat. Using palpation and dissection, I was able to determine that female *O.
maccanii* at this site have a synchronous annual reproductive cycle. This pattern involves
autumn-spring vitellogenesis and a spring-summer gestation of 4-5 months. This pattern is
typical of temperate, southern hemisphere lizards (van Wyk 1989; Ibargüengoytia and Cussac
1996; Jones and Swain 1996; Jones *et al.* 1997) and also agrees with that inferred for female
*O. otagonse* and *O. grande* using palpation and hormone analyses (Cree 1994). As predicted,
this pattern differs to that of *H. maculatus* at this site, in which gestation lasts 14 months with
ovulation during September/October and parturition in October/December. How then, can two
species from the same site have such differing reproductive cycles? What is it that allows *O.
maccanii* but not *H. maculatus* to reproduce annually?

I have investigated one possibility for the difference in reproductive cycles between these two
species. I compared the relative clutch mass (RCM, total mass of neonates relative to maternal
post-partum mass) of *O. maccanii* to existing values for *H. maculatus*. I hypothesised that
female *O. maccanii* would invest less into their young than *H. maculatus*, as a trade-off for
annual reproduction. Although my comparison was not definitive due to a lack of overlap in
body size, it seems that there is no difference in RCM between these two species, or, if
anything, a larger RCM in *O. maccanni*. Therefore, there must be other reasons for the
difference in reproductive cycles, such as life history traits (e.g. clutch size constraints in the
geckos; size at maturity; longevity) and/or temperature availability, both of which are
discussed in Chapter 3. If temperature is the main limiting factor for reproduction in female
*H. maculatus*, it would be worth investigating whether temperature also limits reproduction in
male *H. maculatus* compared to male *O. maccanni*.

As an aside, although I did get equivocal answers on the difference in RCM between *O.
maccanni* and *H. maculatus*, I did have findings that raise other questions. My results showed
that RCM of *Naultinus manukanus*, a diurnal gecko from Stephens Island (Takapourewa),
was higher than that for *H. maculatus*. As clutch size was equal, the difference indicates a
relatively large mass of neonates in *N. manukanus*. This supports my hypothesis that the
relative clutch mass of a diurnal gecko will be larger than that of a nocturnal gecko, based on
differences in body temperature and reproductive cycles. To better understand the limitations
to reproduction it would be valuable to collect data on RCM for a variety of other species. In
particular, I recommend *N. gemmeus* as another diurnal gecko (Wilson and Cree 2003); *H.
maculatus* from Alexandra, as they are annual breeders and are smaller in size than the
population from Macraes Flat (MacAvoy 1976; Cree 1994; Rock 1999); and *O. grande* as a
diurnal skink that overlaps in size with *H. maculatus* (Cree 1994).

Returning to the topic of how *O. maccanni* can be annual breeders while *H. maculatus* are
biennial breeders, one factor that might contribute to allowing female *O. maccanni* to breed
annually is the possibility of starting vitellogenesis during gestation. This is suspected in
*Naultinus gemmeus* from the Otago Peninsula (Wilson and Cree 2003) and *H. maculatus* from
Alexandra (Rock and Cree 2003). There was an increase in diameter of ovarian follicles of *O.
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*maccanni* over gestation; however, it is unknown whether this was pre-vitellogenic or vitellogenic growth. I recommend histology be carried out on the preserved ovaries of females that were in late pregnancy, to determine if vitellogenesis starts during gestation.

According to the plasma progesterone profile that I analysed, it might be feasible for vitellogenesis to start near the end of gestation. One of the suggested roles of elevated progesterone during gestation is to slow the rate of ovarian development, delaying subsequent ovulations (Jones and Baxter 1991; Callard *et al.* 1992; Bonnet *et al.* 2001). In fact, studies on the turtle *Chrysemys picta* have shown progesterone to inhibit vitellogenin synthesis in males (which also have the genetic information and ability to synthesise vitellogenin), and this effect was dose-dependent (Ho *et al.* 1981; Ho 1991). Plasma progesterone levels were highest during mid-pregnancy in female *O. maccanni*, but levels had significantly fallen (although not to basal levels) by late pregnancy, approximately 3-4 weeks before birth. If histology does show vitellogenesis starting before the end of gestation in *O. maccanni*, I would recommend analysing plasma progesterone in females in the final weeks of gestation, and directly prior to birth. This would investigate whether plasma progesterone falls to basal levels prior to parturition, and would increase our knowledge of the role progesterone plays in gestation.

Vitellogenic growth has clearly begun by autumn, however, I am uncertain whether it continues over winter, or whether it ceases during this time and resumes in spring. Deposition of yolk into vitellogenic follicles does occur over winter in *O. zelandicum* (Barwick 1959). Ovary mass remained constant over winter in *Oligosoma* spp. from Alexandra suggesting yolk deposition ceases during this time (MacAvoy 1976). More frequent sampling would be needed to find out whether yolk deposition continues over winter in *O. maccanni* from Macraes Flat. There is a decrease in abdominal fat bodies in female *O. maccanni* between the
start of vitellogenesis (April) and late vitellogenesis/early pregnancy (September). However as winter occurred between these samples, again without more frequent sampling I am unable to conclusively determine whether the decrease in abdominal fat bodies is due to maintenance over winter torpor, the reproductive demands of vitellogenesis, or a combination of both. Abdominal fat bodies of *O. zelandicum* from Wellington districts, New Zealand (which is a warmer climate than Macraes Flat), do not decrease over winter (also when vitellogenesis takes place); instead, they decrease during gestation (Barwick 1959). Fat bodies decreased in mass during both follicular growth and during winter in *Oligosoma* spp. from Alexandra (MacAvoy 1976). It would be useful to know for *O. maccanni*, firstly if vitellogenesis continues over winter, and if it does, whether abdominal fat bodies are needed for both general body maintenance over winter and for the reproductive demands of vitellogenesis. This would be useful in terms of understanding limitations to reproduction in *O. maccanni* and other lizard species from this cold climate. It would also be interesting to investigate mass of abdominal fat bodies in male *O. maccanni* during different times of the year, and to relate any resulting cycles to winter and/or reproduction.

It seems, from the large size of vitellogenic follicles, that *O. maccanni* are relatively lecithotrophic. However, water at least is provided after ovulation, as indicated by total wet mass of ova at late pregnancy being $2.5 \times$ the total wet mass of ova at early pregnancy. Embryos at stage 40 had a considerable amount of growth left to occur before birth. Although these embryos still had large amounts of yolk reserves, I assume it is used up in the final weeks of gestation. As embryos were used for another study (Yeong 2003) I could not determine the dry mass of embryos. Therefore, I cannot describe the relationships between yolk, placenta and embryo development. It is possible that some nutrients are supplied to the embryos via the placenta; however, further studies would be needed to test this. Further
studies could include taking dry weights of both yolk and embryos throughout development; injecting radiolabelled nutrients into females and assaying fetal tissues (Swain and Jones 1997); and comparing chemical composition of newly ovulated ova to neonates (Stewart 1992). Although studies such as these increase our knowledge of the evolution of viviparity, due to the threatened status of many of New Zealand’s lizards, I believe non-invasive studies of reproductive cycles that increase our knowledge for conservation are more important at this stage.

5.2 Comparison of information from this study to other studies on *O. maccanni*

Information on the reproductive cycle of female *O. maccanni* collected throughout this study from Macraes Flat is similar to previous studies on *O. maccanni* and/or closely related species from different areas. Ovulation took place during September in females from the present study and in *Oligosoma* spp. (as *Leiolopis zelandica*, including what is now recognised as *O. maccanni*) from Alexandra, New Zealand (MacAvoy 1976). However, gestation lasts for 4-5 months in females from Macraes Flat, whereas it lasts for only approximately 3 months in females from Alexandra (MacAvoy 1976). This is consistent with the shorter gestation of *H. maculatus* at Alexandra compared to *H. maculatus* at Macraes Flat. This could be due to warmer temperatures at Alexandra compared to Macraes Flat, which could speed up embryogenesis. In fact, differences in gestation length may differ between years in the same population. Patterson (1985) studied *Oligosoma* spp. (as *Leiolopis nigiplantare maccanni*) from the Rock and Pillar and Lammermoor ranges and reported parturition to occur in late January during 1982 and late February during 1983. Patterson (1985) suggested this difference in timing could be due to the exceptionally cold and wet summer of 1982-1983 and may have caused a delay in the maturation of ova in the females.
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Clutch sizes in female *O. maccanni* from Macraes Flat are also similar to that reported in previous studies. The average clutch size in *O. maccanni* from Macraes Flat was estimated from viable oviducal ova of pregnant females and live births in the laboratory from this study, and live births from J. Marshall's study. The average number was 2.9 embryos or offspring (range 1-6 for 35 females). Similarly, Patterson and Daugherty (1990) reported a maximum number of oviducal ova from *O. maccanni* as six (from 21 females as *L. maccanni* from various locations). The mean number of offspring from live births was reported as three from four females (Patterson and Daugherty 1990). MacAvoy (1976) also reported similar figures, with an average clutch size estimated from oviducal ova as 3.06 (range 2-5) in *Oligosoma* species from Alexandra. In all studies, maternal size was correlated with clutch size so that larger females produced more offspring (MacAvoy 1976; Patterson and Daugherty 1990).

5.3 Accuracy of palpation and its applications

Palpation was carried out in the field then again in the laboratory on a subset of *O. maccanni*, which were then dissected. Females were correctly identified by absence of evertable hemipenes. Palpation was accurate in distinguishing among vitellogenic, early-mid-pregnant, late-pregnant, and non-pregnant female *O. maccanni*. It is important to understand the reproductive cycle of lizards so management and conservation plans can be set out as most appropriate. Also, it is useful to know if mature sized females are not reproducing when they should be, so action can be taken to find what is limiting their reproductive output. I suggest that palpation will be accurate in determining reproductive condition of most New Zealand lizards. As plasma progesterone levels showed a relationship with gestation in *O. maccanni*, I recommend profiling plasma progesterone on a subset of lizards to confirm palpation results. However, I acknowledge that this would require extra expertise and a specialist laboratory to
blood sample and analyse the samples using radioimmunoassay, which is also an expensive procedure.

Palpation was most accurate in estimating clutch size in female *O. maccanni* in early-mid pregnancy. This was due to the size and positioning of the oviducal ova. Knowing the average clutch size of a lizard population is important for conservation purposes. For example, clutch size is often a parameter used in population models to monitor the stability or decline of a population. Also, clutch size of captive populations can be compared to wild populations to investigate possible limitations for wild populations in terms of reproductive output. Knowing the clutch size of a lizard on an individual scale can also be important. For example, in studies on temperature-dependent sex determination it is important to know the clutch size of an individual at the start of pregnancy to rule out sex-specific mortality of embryos when birth occurs. For the most accurate estimates in clutch size using palpation, I recommend palpating females in early-mid pregnancy. However, it is important to keep in mind that some females may have unfertilised ova at this stage that are still the same size as and shape of viable ova. Therefore, clutch size of live neonates will be less than that palpated for these females.

In conclusion, the information obtained on the reproductive cycle of female *O. maccanni* is valuable in terms of understanding reproductive constraints, and potentially for conservation. The information on RCM suggests this is not the factor allowing annual reproduction in *O. maccanni*. However, more data on RCM should be collected on the species used in this study, and other species mentioned, to increase our knowledge of reproduction in skinks versus geckos, and diurnal versus nocturnal species. Palpation is a valuable, non-invasive tool accurate in determining reproductive condition of, and clutch size in, female *O. maccanni*. 

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Palpation could be applied to other New Zealand lizard species, especially those that are threatened, with the help of the information collected in this thesis.
References


