Effects of constant incubation regimes on eggs and hatchlings of the egg-laying skink, *Oligosoma suteri*

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Abstract

The conditions under which reptilian eggs are incubated affect survival probability and physiological attributes of the progeny. The egg-laying skink, *Oligosoma suteri*, is the only endemic oviparous lizard in New Zealand. No controlled laboratory incubation had previously been undertaken, and thus no information was available on the requirements for successful captive incubation. I studied the effects of incubation regime on the eggs and hatchlings of *O. suteri* to four months of age. *Oligosoma suteri* eggs (n = 174) were randomly distributed among three constant incubation temperatures (18°C, 22°C and 26°C) and two water potentials (-120 kPa and -270 kPa). Hatching success and hatchling survival were greatest at 22°C and 26°C, with hatchlings from 18°C incubation suffering from physical abnormalities. Incubation regime and maternal influence did not affect sex of individuals, with equal sex ratios occurring from each incubation treatment. Hatchlings from the 22°C and -120 kPa incubation treatments were larger, for most measurements, and warmer incubation temperatures resulted in increased growth rates. Juveniles from 22°C and 26°C and individuals with greater mass per unit length (condition index) sprinted faster over 0.25 m. Sprint speed was positively correlated with ambient temperature. At four months of age sprint speed decreased in 18°C individuals and individuals incubated at 26°C and -270 kPa compared to their performance at one month. The results suggest that the most successful captive incubation regime for *O. suteri* is 22°C and -120 kPa. This study also shows that temperature-dependent sex determination does not occur in *O. suteri*, but that fitness traits are influenced by incubation temperature.

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Table of Contents

CHAPTER ONE
Incubation, sex determination, fitness and the egg-laying skink, *Oligosoma suteri*: introduction and overview

Hatchling survival probability and physiological attributes, such as sex, fitness and growth, are key components in conservation programs of oviparous (egg-laying) reptiles. In New Zealand, tuatara (*Sphenodon*) eggs have been incubated under controlled laboratory conditions to determine the requirements necessary for successful incubation (Thompson 1990). This study also led to the discovery of temperature-dependent sex determination (TSD) in tuatara (Cree *et al.* 1995), which in turn meant that particular incubation regimes could be designed to produce juveniles of both sexes for establishment of new populations (Daugherty 1998).

New Zealand's only other endemic oviparous reptile, the egg-laying skink, *Oligosoma suteri*, has had both its ecology and reproductive biology described (Towns 1975a, b, Whitaker 1968), but no controlled laboratory incubation has been undertaken. Thus, no information is available on the requirements for successful captive incubation, nor the effects of different incubation regimes on physiological attributes of *O. suteri*. In the past, inadequate understanding of incubation regime and its impact on reptile physiology and reproduction has led to poor management. For example, before the discovery of TSD in the loggerhead sea turtle (*Caretta caretta*), conservation methods included incubation of eggs in styrofoam boxes in sheltered locations on beaches (Mrosovsky and Yntema 1980). The cool temperatures experienced by embryos resulted in a male biased sex ratio, thus compromising conservation of the species.

1.1 Effects of incubation regime

In reptiles, incubation temperatures (both constant and variable) have been found to affect incubation period, embryonic survival, sex, size at hatching, morphology, growth rate, locomotor performance, thermoregulatory behaviour and juvenile survival (e.g. Alberts *et al.* 1997, Allsteadt and Lang 1995, Andrews *et al.* 2000, Bull 1980, Choo and Chou 1987, Elphick and Shine 1998). Embryos of reptiles develop faster at warmer temperatures than cooler temperatures, with highest survival at an optimal (usually intermediate) temperature (Christian 1986, Packard and Packard 1987, Plummer *et al.* 1994). Inappropriate incubation temperature may have dire effects on embryos and hatchlings. For example, the snake *Python molurus* develops normally at 30.5°C, but abnormally at 27.5°C, when deformities such as kinking of vertebral column occur (Vinegar 1974).

The availability of water to soft-shelled eggs during incubation also influences numerous aspects of hatchling reptile phenotypes. Most of this work has been undertaken on turtles, but eggs of oviparous squamate reptiles usually have thin flexible shells, with little resistance to water movement from the surrounding environment (Packard *et al.* 1982). Therefore, moisture levels during incubation also affect them. Physiological attributes affected by water potential include incubation time, oxygen uptake of the embryo, embryo survival, hatchling size, growth and locomotor performance (e.g. Cagle *et al.* 1993, Gettinger *et al.* 1984, Miller *et al.* 1987, Morris *et al.* 1983, Packard and Phillips 1994). Water potential was also thought to determine sex (Gutzke and Paukstis 1983), but this has since been discredited (Packard *et al.* 1989, Packard *et al.* 1991).

1.1.1 Sex determination in reptiles

Sex determining mechanisms in animals are varied. In reptiles there are two known mechanisms, genetic sex determination (GSD) and environmental sex determination (ESD). GSD occurs when the sex of the offspring is irreversibly fixed by its genotype (Bull 1980). This includes the XX/XY system, where the male has heteromorphic sex chromosomes, and the ZZ/ZW system, where the female has the heteromorphic sex chromosomes (Russell 1998). ESD occurs when the environment encountered as an embryo determines the sex of the offspring. Temperature-dependent sex determination

is a special case of ESD and is common in reptiles (Bull 1980). The thermosensitive period for sex determination in reptiles occurs between specific stages, usually in the middle third to half of development (Birchard and Reiber 1995, Bull 1981).

The first report of TSD in reptiles was in the agamid lizard *Agama agama* (Charnier 1966). Since then only two other lizard families (Eublepharidae and Gekkonidae) have convincingly demonstrated TSD (Harlow 2000, Viets *et al.* 1994). To date, TSD has only been reported in one species of skink, the viviparous Australian skink *Eulamprus tympanum* (M.B. Thompson pers. comm.). The presence of heteromorphic sex chromosomes in reptiles coincides with genetically determined sex (GSD) and a lack of TSD; GSD is most common in squamates (Viets *et al.* 1994). To date, there is no record of TSD in snakes (Order Squamata), but incubation temperatures do differentially affect embryonic mortality in the snake *Pituophis melanoleucus* (Burger and Zappaloorti 1988). TSD also occurs among crocodiles, tuatara and turtles, although a few species of turtles have distinct sex chromosomes (Cree *et al.* 1995, Ewert and Nelson 1991, Ferguson and Joanen 1982, Mittwoch 1996).

Three patterns of sex ratio have been discovered in response to temperature. These include:

- **Ia (MF):** males at low temperatures and females at high temperatures (many turtle species);
- **Ib (FM):** females at low temperatures and males at high temperatures (alligators, some lizards and *Sphenodon punctatus*);
- **II (FMF):** females at low and high temperatures and males at intermediate temperatures (crocodiles, some lizards, and snapping turtles; Bull 1980, Cree *et al.* 1995, Mittwoch 1996).

Although TSD appears to have evolved many times, its significance and origins are still unresolved (Janzen 1995, Shine 1995).

1.1.2 Biological fitness

Biological fitness is the ability of an organism to transfer its genes to the next generation (Hale *et al.* 1995). Studies of biological fitness should estimate the contribution of individuals, genotypes and phenotypes to the gene pool for each generation. However, it is seldom possible to measure lifetime reproductive success in a single individual or population, even for one generation. Instead performance

correlates of phenotypic traits, such as size, growth and locomotor performance, are frequently used to infer overall fitness of individuals, by their presumed influence on an organism's ability to survive to maturity and thus contribute to the gene pool (Russell 1998).

In oviparous reptile species phenotypic traits such as hatchling size can be influenced by variation in initial egg size, egg quality, incubation conditions or combinations of these factors (Crump 1984, Packard and Phillips 1994). Usually, individuals are larger when incubated at optimal incubation temperatures and wetter water potentials. For example, *Crocodylus niloticus* hatchlings are significantly longer from 31°C incubation temperatures than from 28°C or 34°C (Hutton 1987), and painted turtles (*Chrysemys picta*) are larger when hatched from eggs incubated at wet rather than dry water potentials (Packard *et al.* 1991). Incubation conditions also have long-term effects on post-hatching growth, behaviour and locomotor performance in reptiles (Burger 1989, Joanen *et al.* 1987). For example, individuals of the skink *Bassiana duperreyi* incubated in warm conditions (27°C ± 4°C) are faster than cool incubated siblings (20°C ± 4°C) to 20 weeks of age (Elphick and Shine 1998).

Locomotion based fitness tests are performed to evaluate the ability of young lizards to avoid predators. Greater locomotor performance, such as faster sprint speed, may allow individuals to evade predators more successfully and have greater ability to catch food, which can then increase growth rate and thus size at maturity and reproductive success (Downes and Shine 1999, Froese and Burghadt 1974, Jayne and Bennett 1990). Greater locomotor and competitive ability also increase survival probability of large hatchling lizards (Ferguson and Fox 1984, Ferguson and Joanen 1982, Fox 1978), snakes (Jayne and Bennett 1990), and turtles (Alho *et al.* 1985, Janzen 1993) during the neonatal period compared with small hatchlings in the same cohort.

1.2 The egg-laying skink, *O. suteri*

1.2.1 Phylogeny

There are two families of endemic lizards in New Zealand, the Gekkonidae (geckos) and Scincidae (skinks). Currently 37 extant gecko and 34 extant skink species are known (C. H. Daugherty and R. A. Hitchmough pers. comm.), although this number is likely to increase as more research is carried out. The New Zealand skink genera include eight species of *Cyclodina* and 26 species of *Oligosoma* (C. H. Daugherty and R. A. Hitchmough pers. comm.). The genus *Oligosoma* was formerly classified in *Leiolopisma* (Hardy 1977), but changed in 1995 when it was found that *Oligosoma* species are differentiated both morphologically and genetically from the Australian *Leiolopisma* (Patterson and Daugherty 1995).

Oligosoma suteri is the only endemic oviparous lizard species in New Zealand (Whitaker 1968). Preliminary work suggests that it is phylogenetically nested within the endemic lizards of New Zealand (Smith *et al.* 2001). New Zealand is the only landmass in the Pacific region where there is such a dominance of viviparity (live-bearing) over oviparity in the endemic lizards (Robb 1973).

1.2.2 Ecology and reproduction

Oligosoma suteri are black, grey or brown in colour, with black or dark brown, indistinct spots on the dorsal surface and flanks (Gill and Whitaker 1996). They have grey, pink or orange undersides and can reach a maximum snout-vent length of 108 mm (Gill and Whitaker 1996). Their distribution is confined to the upper North Island, being widespread on north-eastern offshore islands (Thoresen 1967). A limited number also exist at a few mainland sites from Cape Reinga to Port Jackson (Gill and Whitaker 1996, Fawcett 1971). Survival of *O. suteri* exceeds 12 years on offshore islands, but is probably lower on mainland New Zealand due to mammalian predators (Towns and Daugherty 1994, Towns and Ferreira 2001). *Oligosoma suteri* are restricted to boulder or shingle beaches and rocky platforms in the splash zone (Towns 1975a, Whitaker 1973). Although nocturnal, mainly hiding by day in crevices or under piles of seaweed, they are known to bask in captivity (Gill and Whitaker 1996, Towns 1975a).

Female *O. suteri* lay two to five eggs in the summer (mid December to early January) in sand or soil deposits beneath large stones above high tide (Towns 1975a), with some females sharing nests (Gill and Whitaker 1996). Their eggs are oval and leathery, on average 15 mm long and 10 mm wide (Whitaker 1968). Incubation takes up to three months in the wild with eggs swelling by up to a third of the original volume by the time of hatching (Whitaker 1968).

1.2.3 Conservation

Although legally protected both by the Wildlife Act 1953 (Part I: Section 3 – amendment Statutory Regulation 2(1) 1996) and by its occurrence on various island sanctuaries, *O. suteri* is neither rare nor endangered (Towns 1988). However, if *O. suteri* is found to have its sex ratio and fitness influenced by incubation regime, then this could have major implications on their future survival. As their distribution is limited and their habitat restricted, demographic and environmental stochasticity could vary their population structure between beaches, islands or with climate change, with possibly harmful long-term consequences to the species. Therefore, the study of incubation regime effects on sex ratio and fitness is important in the ongoing conservation of populations, and ultimately the species.

1.3 Study objectives

This thesis investigates the effects of different incubation temperatures and water potentials on physiological attributes of *O. suteri* eggs and hatchlings. In particular, I determine the conditions required for successful artificial incubation, focusing on incubation period, hatching success, hatchling morphology and sex (Chapter 2), as well as the differences in fitness correlates among different treatment groups, focusing on sprint speed, hatchling size, growth and survival (Chapter 3). Chapters 2 and 3 are written as separate manuscripts to be submitted for publication, so some repetition of general information occurs. A synopsis of general findings and recommendations for future research are detailed in Chapter 4.

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CHAPTER TWO
Incubation regime affects morphology and incubation period but not sex of the egg-laying skink, *Oligosoma suteri*

2.1 Introduction

The egg-laying skink, *Oligosoma suteri,* is the only endemic, oviparous lizard species in New Zealand (Whitaker 1968). It is restricted to beaches, mainly on north-eastern offshore islands (Thoresen 1967, Towns 1975a, Whitaker 1968), and nests under large stones at the top of the shore (Towns 1975a). Reptile eggs are affected by incubation conditions, influencing many physiological factors, including incubation period, hatching success, hatchling phenotype and sex (Packard and Phillips 1994). Incubation regime during embryonic development can thus affect the life history, survival and reproductive fitness of individuals, populations and ultimately species of reptiles.

Reptile embryos develop faster at warmer temperatures than cooler temperatures (Packard and Packard 1987), with highest survival at an optimal temperature (Christian 1986, Plummer *et al.* 1994). Inappropriate incubation temperature may have dire effects on embryos and hatchlings. For example, a constant incubation temperature of 27.5°C produces skeletal abnormalities in embryos and hatchlings of the snake *Python molurus* (Vinegar 1974).

Incubation temperature also affects the sex of many reptiles (Bull 1980, Mittwoch 1996), a phenomenon termed temperature-dependent sex determination (TSD). For example, female tuatara (*Sphenodon punctatus*) are produced at low (18°C) incubation temperatures and males at warm (22°C) temperatures (Cree *et al.* 1995). To date, TSD has been reported in only one species of skink, the viviparous Australian skink, *Eulamprus tympanum* (M.B. Thompson pers. comm.). The presence of heteromorphic sex chromosomes in reptiles coincides with genetically determined sex (GSD), and thus a lack of TSD, and is most common in squamates (Bull 1980).

Water potential of the incubation medium can also affect the physiology of embryo and hatchling reptiles. For example, an increase in moisture content of incubation substrate increases incubation period and size at hatching in the snapping turtle, *Chelydra serpentina* (Morris *et al.* 1983).

The aim of this chapter is to investigate the effects of different constant incubation temperatures and water potentials during captive incubation on physiological attributes of *O. suteri*. In particular, I sought to determine the conditions required for successful artificial incubation, focusing on length of incubation, change in egg mass, hatching success, hatchling morphology and sex of hatchlings.

2.2 Methods

2.2.1 Study area

Gravid adult females were collected from Green Island (Figure 2.1), a small (3 ha) island in the Mercury Island group, 6 km East of the Coromandel Peninsula, New Zealand (36°39'S, 175°51'E, McKenzie 1995, Towns *et al.* 1990). The island has never had introduced mammalian predators and has remained largely unmodified (Towns *et al.* 1990), and thus has one of the highest known concentrations of *O. suteri* in New Zealand (Whitaker 1973). The Mercury Islands receive high but variable annual rainfall and experience warm humid summers, reaching 25°C or greater for 10-20 days of the year, with a mean annual temperature of 14-15°C (Kirkpatrick 1999).

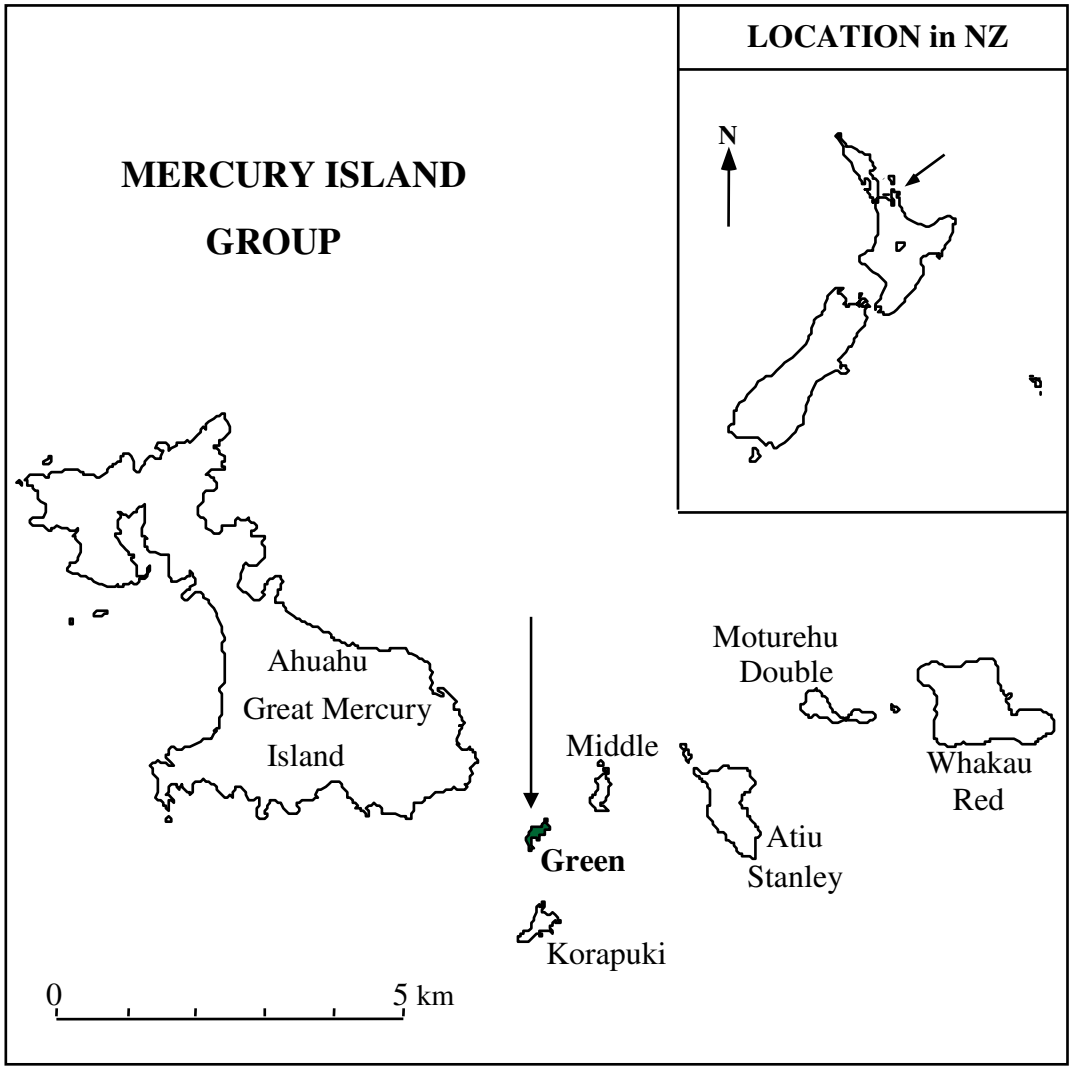

Figure 2.1 Location of the Mercury Island Group in New Zealand (insert) and Green Island (shaded) within the Mercury Island Group.

2.2.2 Collection of gravid females

Fifty eight gravid females were collected, using pitfall traps and night searches, between 30 November and 7 December 1999. The traps consisted of 30 white paint pails (4 L), 6 brown buckets (5 L), and 10 metal paint tins (4 L) and were set just above high tide amongst seaweed mats on the boulder beach. Square plastic covers (5 x 250 x 250 mm) were erected 30 mm above traps to protect skinks from predators, such as tuatara (*Sphenodon punctatus punctatus*). Traps were baited with a seafood based cat food, canned corned beef or fresh salami, and were set half an hour before dusk each evening. Traps were cleared of lizards each morning between 0630 and 0700 h to ensure they did not overheat in the sun.

Females were identified as gravid by the observation of eggs, visible through the abdominal wall as lighter marks. They were weighed using a Pesola™ balance ± 0.1 g, and snout-vent length (SVL) and vent-tail length (VTL) were measured to 0.5 mm with a clear, 300 mm plastic ruler. Individuals without natural toe loss were uniquely marked by toe-clipping (Appendix I) for identification. Females were kept in ventilated 2 L plastic containers (two per container), with soil and leaves for substrate, and transported to Victoria University of Wellington (VUW) in cloth bags (two per bag).

2.2.3 Husbandry

Females were individually housed in identical conditions in transparent plastic boxes (New Ocean™ Plastic No. 838, 215 x 330 x 110 mm, 7 L) with a square of wire mesh (165 x 120 mm) on the lid allowing airflow. Boxes contained moist, non-fertilised potting mix in the bottom (at least 20 mm deep), pieces of pine (*Pinus radiata*) bark for shelter, and water provided *ad libitum* in a round plastic bowl (30 mm deep, 70 mm diameter). To allow for behavioural thermoregulation, skinks had a choice of temperatures, ranging from room temperature (> 14.5°C) to 30°C (provided by a strip heater along the back of each box). The boxes were stacked on metal shelves with simulated natural lighting (Duro-test® True-lite® power twist) suspended from the ceiling on a 12L:12D light cycle (lights on at 0600 h). Females were fed three times a week on crickets (*Teleogryllus commodus),* slaters (*Porcellio* spp.), houseflies (*Musca domestica*) or blowflies (Family: Calliphoridae), periodically laced with vitamin supplement powder (Villavet calcipup). Enclosures were checked twice daily for eggs

until all females had oviposited (21/12/1999 to 21/01/2000). The skinks were weighed to 1 mg on a Sartørius top loading balance immediately post-oviposition, after which they were returned to Green Island.

2.2.4 Incubation of eggs

One hundred and seventy four eggs from 55 clutches were incubated. The clutches were split, and eggs randomly assigned among three incubation temperatures (18°C, 22°C and 26°C) and two water potentials (-120 kPa and -270 kPa). Each egg was assigned a unique number, written on the top surface with a soft (4B) graphite pencil, to enable maintenance of egg orientation throughout incubation. Conservation and cultural values associated with *O. suteri,* and the areas it inhabits, makes egg collection of this species difficult. Therefore, out of the 214 eggs laid, 38 eggs were contributed to another study outside the scope of this thesis (Stewart, J. R., M. N. Hutchinson and M. B. Thompson, unpub. data), and two eggs were sacrificed immediately after oviposition (Appendix IIa) to enable the embryonic stage at oviposition to be determined. For these studies, eggs from clutches of two, one egg from clutches of four, and two eggs from clutches of five were taken. Thus, three eggs were available from most females for clutch assortment within incubation regimes.

In the absence of data from natural nests, incubation temperatures were selected over a range of temperatures to which eggs were predicted to be exposed in the wild. Incubation temperatures were accurate to ± 0.3°C, as recorded using data loggers in the incubators (StowAway TidbiT Temp Logger, Onset™ Computer Corporation, Massachusetts, USA).

The eggs were initially placed in separate plastic cups (50 mm deep x 55 mm diameter) and half buried in vermiculite. The cups were completely covered with transparent cling-wrap to prevent moisture loss. However, when some eggs began to dehydrate in the cups (12 January 2000), the eggs were reassigned to random positions in 2 L plastic containers (8 to 13 eggs/container and 2 to 4 containers/treatment). The location of egg boxes (and cups) was altered daily to compensate for possible temperature gradients within incubators.

Water potential of the medium grade vermiculite used as an incubation medium was achieved by mixing 100 g of vermiculite with 96.4 ml (-120 kPa) or 32.6 ml (-270 kPa) of water. Two different water potentials were chosen, as eggs of oviparous squamate reptiles usually have thin flexible shells with little resistance to water movement from the surrounding environment, and are thus influenced by moisture availability (Packard *et al.* 1982). The egg box containing moist vermiculite was weighed without eggs or a lid, and this weight maintained by addition of distilled water every seven days to compensate for small losses from the container and uptake by the eggs. Eggs were weighed weekly to 1 mg on a Sartørius top loading balance and a sub-sample candled (by shining a cool heat, fibre-optic light source; Schott Mainz™, KL 150B) to observe viability and development of the embryos**.**

A Q_{10} for incubation period was calculated from incubation periods at 22°C and 26°C (2.46) and used to predict the incubation period for 18°C eggs (105 days). When the 18°C eggs had not hatched 30 days after the predicted day of hatching (4th April 2000), three eggs were dissected and the embryos staged. The rest were randomly assigned to either 18°C or 22°C (4th May 2000) for the remainder of incubation (10 to 29 days).

2.2.5 Sex identification of juveniles

After hatching, juveniles were held in a 2 L plastic container for three days before sex identification was attempted by eversion of hemipenes (Harlow 1996). Identification of sex involved holding the skink in one hand with a thumb on its pelvis, and rolling the end of a small plastic paintbrush up its tail (Figure 2.2). I found this method to be easier than a process described by Harlow (1996), and it had the added benefit of not requiring two people or cooling of the juvenile beforehand. As there can be uncertainty involved in diagnosis of females simply from negative results (DeNardo 1996), gonadal sex was confirmed with histological analysis of animals that died naturally. As this species is protected in New Zealand, juveniles could not be sacrificed for my research.

Fully developed embryos and juveniles that died were fixed, embedded, sectioned to 7 µm, stained (Humason 1979) and mounted (Methods in Appendix II). The skink sections were observed under a compound microscope (Zeiss); the right and left sides of animals were viewed from the dorsal surface.

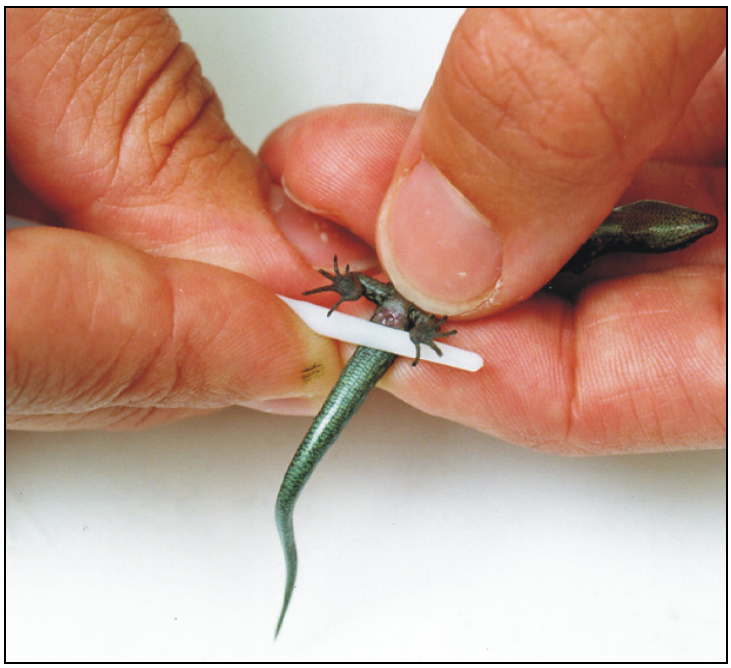

Figure 2.2 Method of hemipene eversion used in this experiment.

2.2.6 Data analysis

Data were analysed using the statistics packages SYSTAT, SPSS and S-plus. All data were tested for normality using exploratory analyses on the statistics program SPSS, and statistical significance was assumed at $p < 0.05$. Data are expressed as mean ± standard error (SE), unless otherwise stated.

2.2.6.1 Eggs

Clutch effects were not investigated as all clutches had five eggs or fewer, too few for powerful statistical tests (pers. comm. Edith Hodgen). However, the effect of maternal size (SVL) on mean initial egg mass of all eggs in a clutch (mean initial egg mass) and number of eggs in a clutch (clutch size) was tested using ANOVA on SPSS.

Only those eggs that hatched without assistance (versus those cut open) were used for incubation analyses. The effect of changing from cups to boxes during incubation versus only incubating in boxes was tested by a MANOVA, with a nested water potential and temperature design, on SYSTAT. The effect of both incubation treatments and initial egg mass on incubation period was assessed using a univariate general linear model (GLM) on SPSS with incubation treatments as independent factors, days of incubation as the dependent variable, and initial egg mass as a covariate.

The effect of incubation regime and initial egg mass on change in egg mass over the incubation period was assessed using ANOVA on SPSS, with incubation treatments as independent factors, change in egg mass as the dependent variable and initial egg mass as a covariate. The effect of incubation regime and initial egg mass on hatching success was assessed using logistic regression models on S-plus with the independent factors initial egg mass and incubation treatments (incubation temperature and water potential as categorical data), and the dependent variable of probability of hatching (1 = hatched, 0 = did not hatch). Akaike information criteria were used to determine the best-fit model and which independent factors did not contribute to hatching success (McCallum 2000). Chi-squared tests (χ^2) were used to compare models. As 95% confidence intervals can take the upper limit of probability above one, and probability of hatching cannot be greater than one (1 = hatched), 95% profile likelihood intervals were used.

2.2.6.2 Sex ratio

Sex ratios were analysed for deviation from a 50:50 ratio for each incubation treatment, using the χ^2 distribution (Sokal and Rohlf 1981).

2.3 Results

There were no significant differences in incubation period and hatching success between individuals incubated only at 18°C and those that had incubation temperature increased to 22°C near the end of incubation. For example, incubation period for 18°C only was 140.0 ± 1.9 days and for those increased to 22°C for the final 10 to 29 days of incubation it was 136.9 ± 2.1 days. Therefore, all parameters for these individuals were combined for further analyses. Similarly, there was no significant influence of changing from cups to boxes and no box affect, which allowed for removal of these parameters for the remainder of the analyses. Results from all statistical tests, significant or otherwise, are presented in Appendix IIIa.

2.3.1 Eggs

The 58 females laid a total of 214 eggs, with clutch sizes varying from two to five eggs (mean 3.6 ± 0.1). Between 27 and 31 eggs were assigned to each incubation regime, two eggs were dissected to determine the embryonic stage at oviposition, and the remaining 38 eggs went to the study described in section 2.2.4. Egg fate was mainly influenced by incubation temperature, with some fully developed embryos failing to hatch at 18°C (Table 2.1). Incubation by the cup method caused dehydration, especially at the warmest, driest incubation regime, whereas no eggs dehydrated in the 2 L plastic containers. No dehydrated eggs hatched successfully.

Table 2.1 Distribution of eggs amongst the incubation treatments, detailing eggs that fail to develop, eggs that fully develop and eggs that hatch (WP = water potential, T = temperature).

WP (kPa)	T (°C)	Egg Distribution	Egg Failed		Full Development	Hatched Successfully
			Mouldy	Dehydrated		
-120	**18**	27	2	1	24	19
	22	27	1	1	25	25
	26	33	4	3	26	26
-270	**18**	28	0	0	28	18
	22	28	1	2	25	25
	26	31	1	7	23	23
Total		174	9	14	151	135

The two eggs dissected at laying contained stage 32 embryos, according to the staging series for *Lacerta vivipara* (Porter 1972). Paddles were distinctly differentiated on each limb, the eye was pigmented and prominent, and a distinct outline of the parietal region and genitalia was apparent (Figure 2.3).

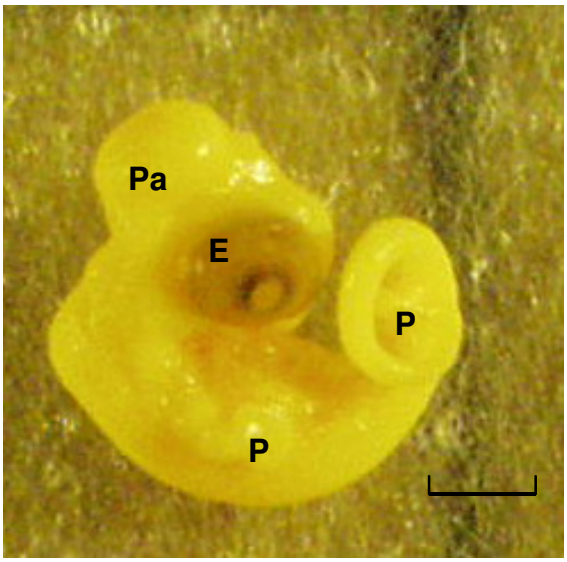

Figure 2.3 *O. suteri* embryo present in egg at oviposition, stage 32 (E = eye, P = paddle, Pa = parietal region - genitalia are behind tail). Scale bar is 1 mm.

The size (SVL) of the mother had no effect on mean egg mass of the clutch ($F = 0.827$, $df = 1$, $p = 0.367$), but did have a statistical effect on clutch size ($F = 5.981$, $df = 3$, $p = 0.001$), with females that oviposited five eggs significantly larger than those that oviposited two to four eggs (Table 2.2). Clutch size was not correlated with mean egg mass of the clutch ($F = 1.085$, $df = 3$, $p = 0.363$).

Table 2.2 Mean maternal snout-vent length (SVL) and egg mass for each clutch size.

Clutch Size	**Mean SVL ± SE (mm)**	**Mean Egg Mass ± SE (g)**	**No. Clutches**
2	96.7 ± 1.2	1.042 ± 0.08	3
3	94.3 ± 0.6	0.935 ± 0.02	20
4	97.3 ± 0.6	0.944 ± 0.02	31
5	101.0 ± 2.0	0.906 ± 0.06	4

Incubation period was affected by temperature ($F = 5715.287$, $df = 2$, $p < 0.001$), but not water potential ($F = 0.497$, $df = 1$, $p = 0.482$), initial egg mass ($F = 0.577$, $df = 1$, $p = 0.449$), or any combination of these three factors. Eggs took longer to hatch at cooler temperatures; eggs at 18°C had the longest incubation period (mean = 137.9 ± 0.9 days). Eggs at the two warmer temperatures (22°C and 26°C) took, on average, 73.8 ± 0.5 days and 51.5 ± 0.3 days to hatch, respectively (Figure 2.4).

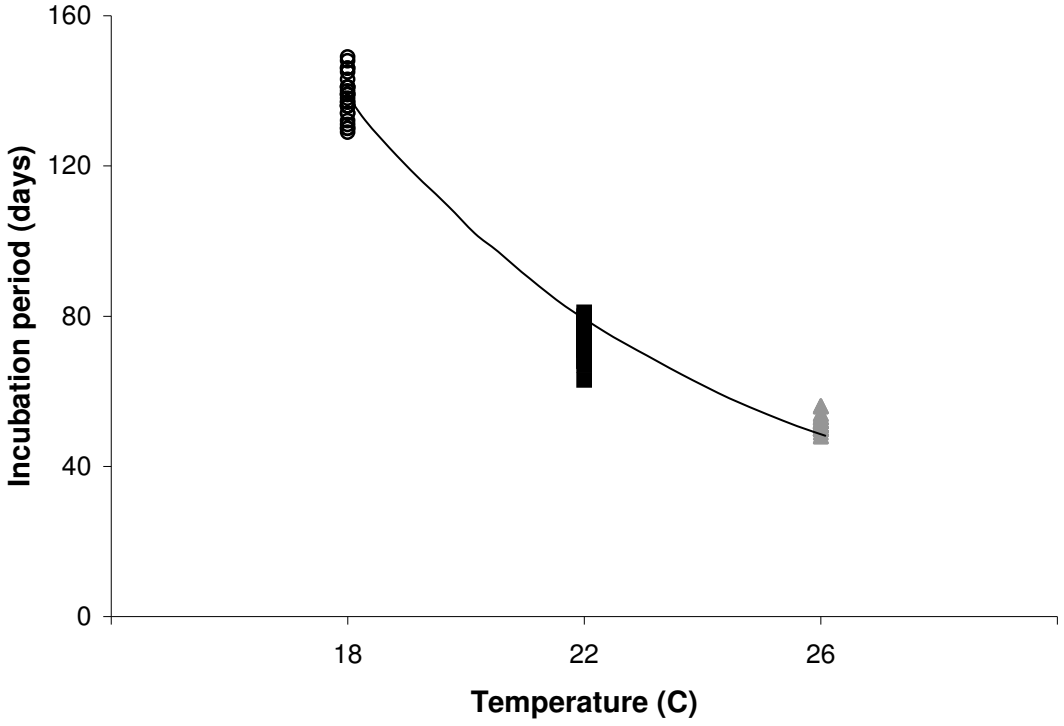

Figure 2.4 Incubation period (days) for each incubation temperature (○ = 18°C, ■ = 22°C, ▲ = 26°C). Note that 18°C includes individuals that completed the last part of incubation at either 18°C or 22°C. The quadratic equation best fitting the data is $y = 1.3181x^2 - 68.843x + 950.37$ ($R^2 = 0.9889$).

Change in egg mass during incubation was significantly influenced by incubation temperature ($F = 8.551$, $df = 2$, $p < 0.001$, Figure 2.5), water potential ($F = 34.150$, $df = 1$, $p < 0.001$) and initial egg mass ($F = 5.819$, $df = 1$, $p = 0.017$), but not by a combination of the two incubation factors ($F = 1.667$, $df = 2$, $p = 0.192$). Change in egg mass was greater for incubation at 18°C, the wetter water potential (-120 kPa) and larger eggs.

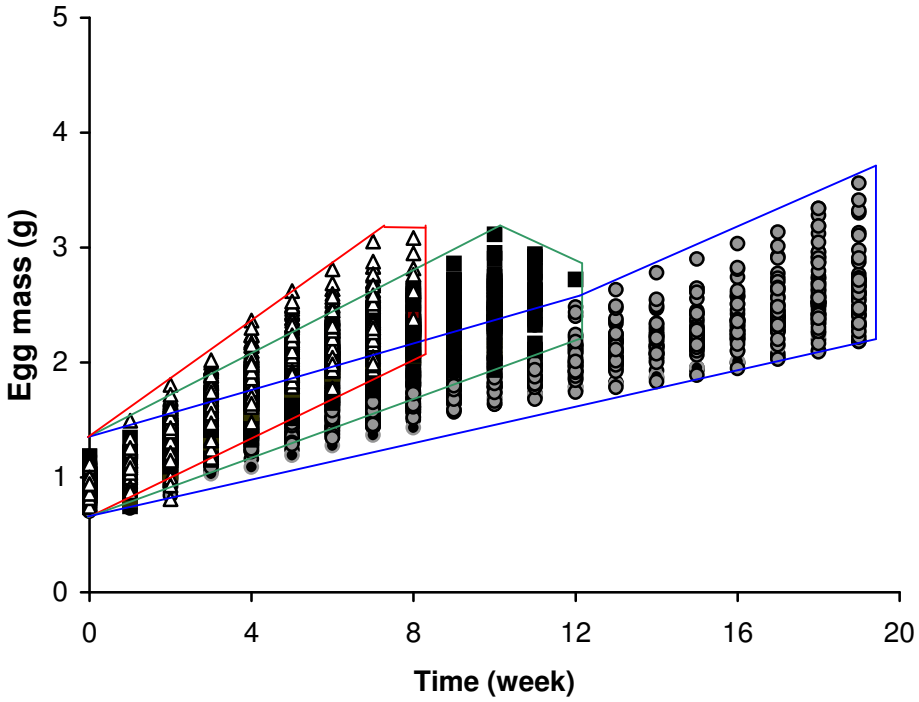

Figure 2.5 Change in egg mass over incubation period for each incubation temperature. The red line outlines 26°C (Δ), green 22°C (■) and blue 18°C (●). Note that 18°C includes individuals that completed the last part of incubation at either 18°C or 22°C.

2.3.2 Hatching success and hatchling morphology

Hatching success was significantly influenced by incubation temperature ($\chi^2 = 8.020$, df = 171,2, p = 0.018) with higher hatching success occurring at the two warmer (22°C and 26°C) incubation temperatures (Figure 2.6). Hatching success was not significantly influenced by water potential ($\chi^2 = 0.828$, df = 172,1, p = 0.363), initial egg mass ($\chi^2 = 0.983$, df = 172,1, p = 0.322), or any combination of these three factors.

Hatchlings from 22°C and 26°C had similar patterning to wild individuals (grey-brown colour with indistinct spots on the dorsal surface and flanks), straight tails and correctly orientated feet and legs. Hatchlings incubated at 18°C (n = 37) had uniform, dark-brown colouration and physical abnormalities; tails were bent (100%, n = 37), front feet paddle-like (91%, n = 34), the rear feet flattened (91%, n = 34), and sometimes the rear legs were also bent backwards at the ankles (11%, n = 4, Figure 2.7).

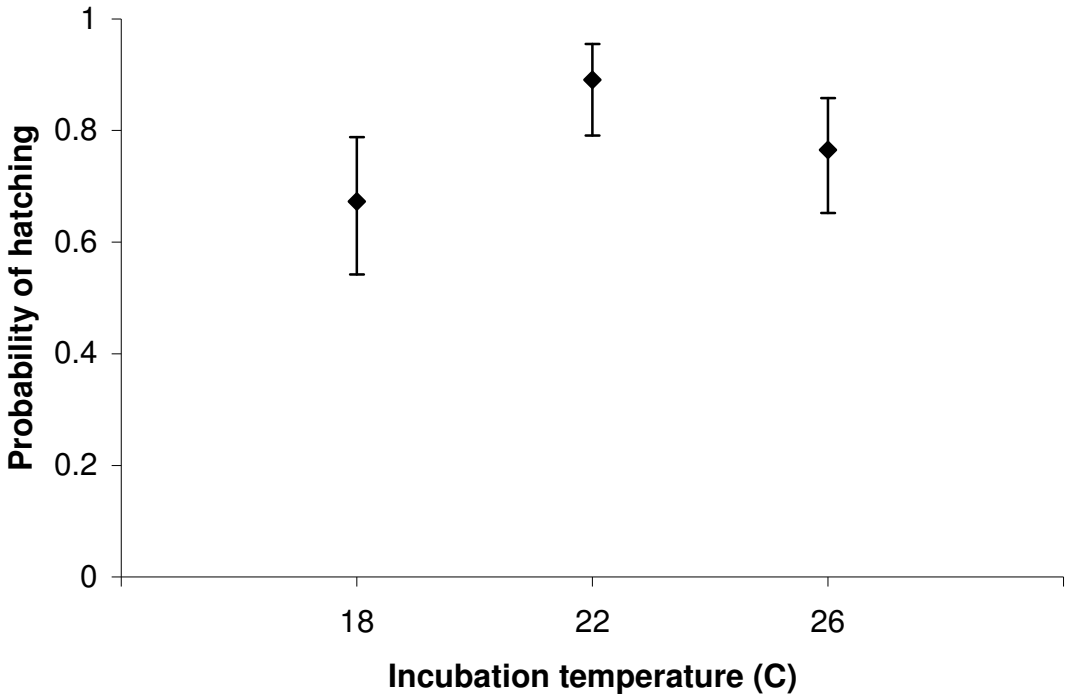

Figure 2.6 Probability of hatching at each incubation temperature ± profile likelihood intervals. Note that 18°C includes individuals that completed the last part of incubation at either 18°C or 22°C.

Figure 2.7 Abnormal phenotype of juveniles incubated at 18°C (upper and lower skinks), showing uniform, dark-brown colouration, bent tails, paddle-like front feet and flattened rear feet, compared to the normal phenotype of a 26°C (centre skink) incubated juvenile.

2.3.3 Identification of hatchling sex

Hemipene eversion allowed hatchlings to be classified into four groups (Tables 2.3a and b). Males (n = 77) had large white projections (Figure 2.8a), while putative males (n = 48) had smaller pink projections (Figure 2.8b). Females (n = 5) had no projections visible (Figure 2.8c), whereas putative females (n = 21) had very small, pink projections (Figure 2.8d).

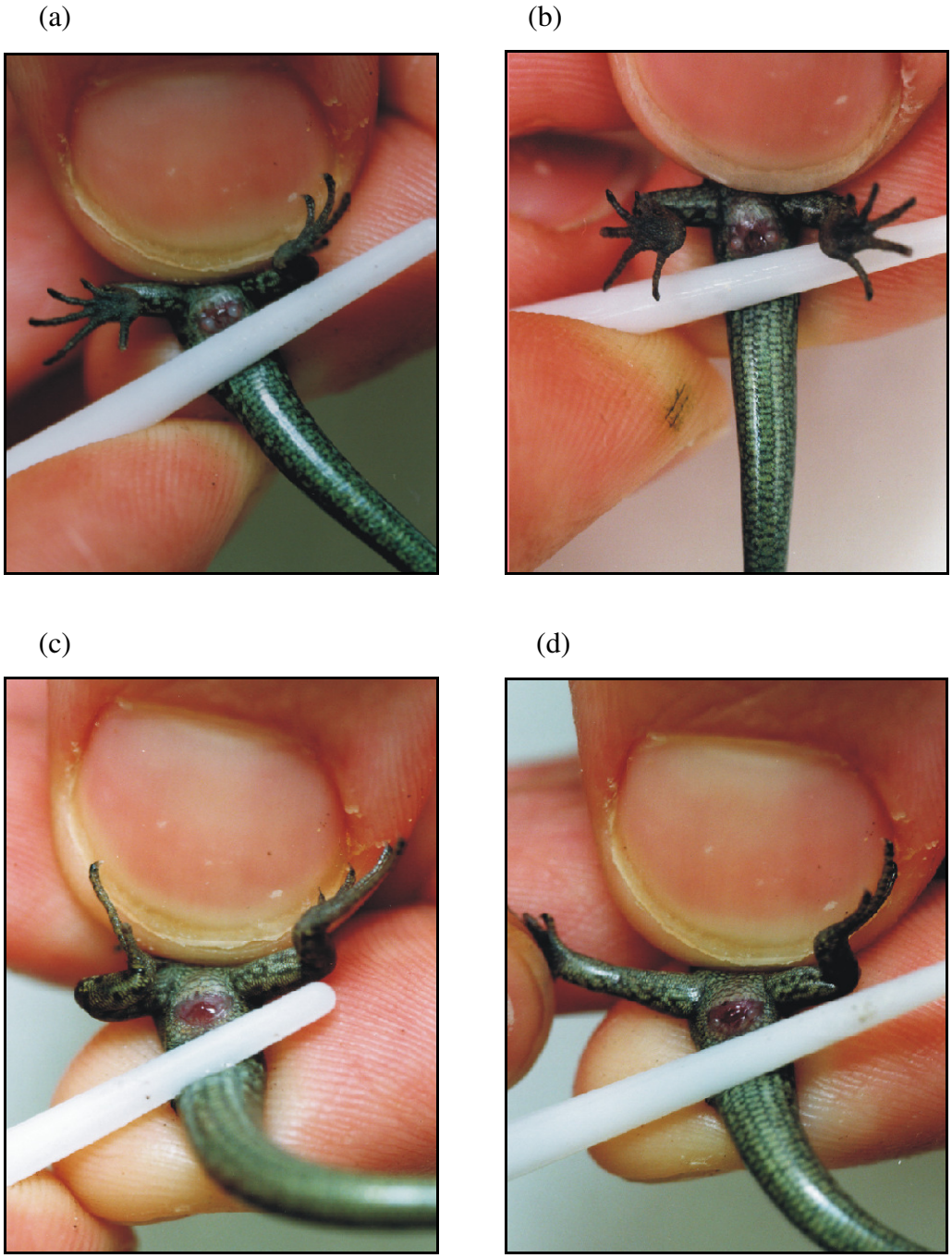

Figure 2.8 Cloacal area of juvenile *O. suteri* during hemipene eversion: (a) male, (b) putative male, (c) female, (d) putative female.

Table 2.3 Number of individuals in each morphological class with corresponding morphological traits for each incubation temperature (a) and water potential (b).

(a)

Initial Morphological Classification	Morphological Trait	Temperature (°C)			Total
		18	22	26	
Male	Large, white	27	25	25	77
Putative male	Small, pink	17	17	14	48
Female	None	0	2	3	5
Putative female	Very small, pink	8	6	7	21

(b)

Initial Morphological Classification	Morphological Trait	Water Potential (kPa)		Total
		-120	-270	
Male	Large, white	37	40	77
Putative male	Small, pink	25	23	48
Female	None	2	3	5
Putative female	Very small, pink	11	10	21

Histological examination showed that all individuals with large white projections had testes; those with small pink, very small pink or no projections had ovaries. As a result, all putative males and putative females defined by eversion of hemipenes were reassigned as females. Among the 21 individuals that had their gender verified by histology, all were diagnosed correctly. Gonad shape was not a good predictor of sex, but cell placement and cell characteristics were.

Ovary characteristics

Most females possessed two ovaries and two müllerian ducts (Figure 2.9a), and lacked any detectable male characteristics. A müllerian duct was not detected in one female on the right side and in a second female on the left; both females had two ovaries. One female lacked an ovary on the left side but still had a müllerian duct; the mesenephric kidney on the left side was also small. All ovaries had large lacunae in the medulla and

a well-developed cortex (Figure 2.9b) and naked oocytes were present in some older individuals. The right ovary was anterior to the left in all specimens.

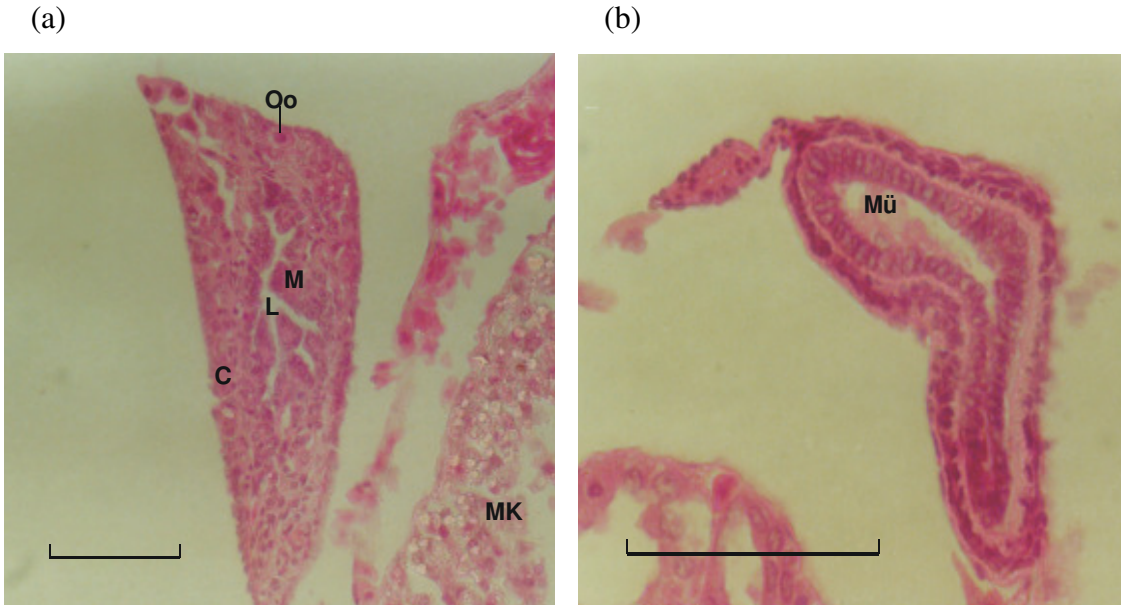

Figure 2.9 Transverse sections of female gonad characteristics in juvenile *O. suteri:* (a) ovary, (b) müllerian duct. (C = cortex, L = lacunae, M = medulla, MK = mesenephric kidney, Mü = müllerian duct, Oo = oocyte). Scale bars are 50 µm.

Testis characteristics

All males possessed two testes and lacked any discernible female characteristics. The right testis was anterior to the left testis in all specimens. Most testes had a relatively well-developed medulla, with developing tubules and some germ cells present (Figure 2.10a). The cortex was mostly poorly developed. A wolffian duct could only be located in one male (Figure 2.10b).

2.3.4 Sex ratio

Sex ratio did not deviate significantly from 50:50 (Table 2.4) at any incubation temperature ($\chi^2 = 0.087$, df = 2, χ^2 statistic = 5.991) or water potential ($\chi^2 = 0.241$, df = 1, χ^2 statistic = 3.841). Sixty nine percent of clutches produced both male and female hatchlings.

(a) (b)

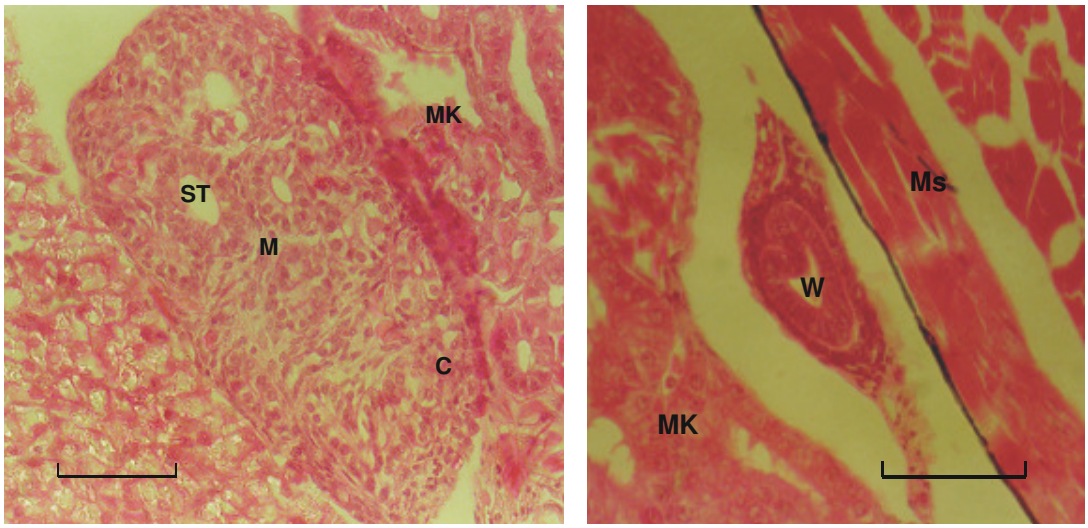

Figure 2.10 Transverse sections of male gonad characteristics in *O. suteri*; (a) testis, (b) wolffian duct. (C = cortex, M = medulla, MK = mesenephric kidney, Ms = muscle, ST = seminiferous tubule, W = wolffian duct). Scale bars are 50 µm.

Table 2.4 Number of males and females from each incubation regime.

		No. Males (%)	**No. Females (%)**
Temperature (°C)	**18**	27 (52)	25 (48)
	22	25 (50)	25 (50)
	26	25 (51)	24 (49)
Water potential (kPa)	**-120**	40 (51)	38 (49)
	-270	37 (51)	36 (49)
	Total	77	74

2.4 Discussion

A constant incubation temperature of 18°C is not optimal for *O. suteri* egg incubation, but 22°C and 26°C and water potentials of -120 kPa and -270 kPa produce healthy hatchlings. Incubation period increases with decreasing incubation temperature; egg mortality is greatest at 18°C and least at 22°C. Incubation regime and maternal influence do not affect sex of individuals, so sex is probably genetically determined at the chromosomal level.

2.4.1 Maternal influence

At oviposition embryos were at stage 32 (Porter 1972). Most oviparous squamates retain eggs *in utero* for half of the total period of embryonic development, compared with other reptiles, which usually only briefly retain eggs in the oviduct, with the usual stage of laying at 30 (Shine 1983). Thus, *O. suteri* oviposit 2 stages later than most other squamate reptiles.

No relationship was evident between size of females and mean egg mass, as also occurs in other reptiles, such as the Australian rock dragon *Ctenophorus decresii* (Harlow 2000). The number of eggs laid in a clutch by many female reptiles increases with the size of the individual, as more room is available in the female for eggs (e.g. tuatara, Cree *et al.* 1991; European lacertid lizards, in den Bosch and Bout 1998). Larger *O. suteri* females in this study also, on average, produced more eggs, which is in contrast to the findings of Towns (1975b). However, it is important to note that some larger females did have small clutch sizes, which may be due to the amount of resources available to them during vitellogenesis. The presumed trade-off between the number and size of offspring a female produces is a fundamental tenet of life history theory and is a general feature of reptilian reproduction (Sinervo 1990, 1994, Smith and Fretwell 1974). It is generally thought that any given female has limited resources available for each reproductive event and cannot increase the number of offspring (fecundity) without decreasing survival and possible fitness of offspring (Jayne and Bennett 1990, Sinervo and Doughty 1996).

2.4.2 Incubation period, eggs and hatching success

An increase in incubation period as incubation temperature decreases is a common phenomenon in oviparous reptiles (e.g. Angilletta *et al.* 2000, Jensen 1982, Lang *et al.* 1989, Lewis-Winokur and Winokur 1995, Thompson 1990). Incubation period of *O. suteri* eggs also increased as temperature decreased, although incubation period is not influenced by the final 10 to 29 days of incubation. The turtle, *Chelydra serpentina* also has a rate of development during the final weeks of incubation independent of environmental temperature (Ynetma 1978).

Change in egg mass during incubation is influenced by incubation conditions. For example, wetter water potential and cooler temperatures increase mass change in eggs of the painted turtle *Chrysemys picta* (Gutzke *et al.* 1987) and bull snake *Pituophis melanoleucus* (Gutzke and Packard 1987). The same pattern was found in *O. suteri*, where wetter water potential (-120 kPa) and cooler incubation temperature (18°C) resulted in overall heavier final egg mass. This is due to more time available during incubation to take up water (Packard and Phillips 1994). Initial egg mass also influenced hatching egg mass, probably due to increased surface area available in larger eggs for water exchange.

Hatching success of *O. suteri* was influenced by incubation temperature. Constant incubation at 18°C resulted in low hatching success and physical abnormalities, such as bent tails. Low hatching success at sub-optimal temperatures occurs in other oviparous reptiles, such as *Alligator mississippiensis*, which develops fully but fails to hatch at 28°C, and fails to develop at 36°C (Lang and Andrews 1994). Physical abnormalities due to low temperature stress also occur in other squamates. For example, the snake *Python molurus* develops normally at 30.5°C and abnormally at 27.5°C, with deformities such as kinking of the vertebral column occurring (Vinegar 1974). A similar trend is found in the bull snake (*Pituophis melanoleucus*), where low hatching success occurs at low (22°C) incubation temperatures and a significantly greater number of abnormalities at high (32°C) temperatures (Gutzke and Packard 1987).

2.4.3 Sex ratio

The thermosensitive period for sex determination in most reptiles with TSD occurs in the middle third to half of embryonic development (Birchard and Reiber 1995). Embryonic development in *O. suteri* takes five months, with eggs retained in the oviducts for the first two months (Towns 1975b). Thus, part of the thermosensitive period for sex determination occurs within the mother when the sexual characteristics are beginning to form. However, both sexes were present in most clutches, indicating that the mother did not selectively influence embryonic sex before oviposition. Moreover, sex ratio of hatchlings was not significantly different from 50:50 in any incubation regime, so sex was probably genetically determined. In reptiles, genetically determined sex is most common in squamates, especially in skinks (Janzen and Paukstis 1991), with TSD found in only one skink tested to date - the viviparous Australian skink, *Eulamprus tympanum* (M. B. Thompson pers. comm.).

2.4.4 Sexual characters

Reptiles do not possess external genitalia, and many, such as *O. suteri*, lack external secondary sexual dimorphism (DeNardo 1996, Towns 1975b). In snakes and lizards hemipenes are located in the cloaca of males, enabling sex to be determined by manual eversion (DeNardo 1996). There are difficulties associated with hemipene analysis, including diagnosis of females from negative results, and the appearance of glands in some females (Harlow 1996). Thus, the small pink projections from the cloaca in some female juvenile *O. suteri* could be glands.

Gonad shape is usually a helpful determinant in sex identification of reptiles, with testes usually thin and elongate and ovaries ovoid (Fox 1977). The shape of gonads was not helpful in identifying the sex of *O. suteri* individuals. Most specimens available for histological analysis were from embryos or hatchlings under 1 month old, and the gonads were probably still differentiating (Fox 1977). Undetectable müllerian and wolffian ducts were possibly lost during preparation of histological material, which may also explain the individual without a left ovary. It is also possible that the ovary was lacking, as occurs in some other reptiles such as the gecko, *Eublepharis macularius* (Bull 1987). However, most juveniles that died in this study suffered from external physical abnormalities, and possibly also internal abnormalities, which could also account for missing structures. The position of testes and ovaries (right gonad

anterior to the left) is consistent with results found by Towns (1975b), and in other lizards (Fox 1977).

2.4.5 Ecological implications

Reptilian development, hatching success and hatchling morphology are influenced, often detrimentally, by deviation of incubation temperature from an optimum. Physical abnormalities occur in *O. suteri* individuals incubated at constant 18°C, which is probably why *O. suteri* have a restricted northern distribution. Current global warming predictions estimate an increase in mean global temperature of 1.4°C to 5.8°C by 2100 (IPCC 2001), which may increase nest temperatures in some populations to the upper lethal limit. Potentially the mainland populations of *O. suteri* could extend their range southward if global temperatures increase and suitable boulder beach habitats are available. However, data on natural nest temperatures are needed before effects of increased global temperatures can be ascertained.

An increase in global temperature (that does not reach upper lethal limits) could be advantageous, decreasing the incubation period and thus affecting timing of hatchling emergence in the wild. Earlier hatchling emergence would increase the time available to hatchlings for growth before hibernation (May to August, Towns 1975a), which could lower the time until maturity, and thus first reproduction, is reached.

2.4.6 Conclusion

The data presented in this chapter confirms that *O. suteri* physiology is influenced by incubation temperature, as is that of all oviparous reptiles, as well as providing a successful captive incubation regime for *O. suteri*. The sex of *O. suteri* is probably genetically determined, as is found in all but one other skink to date (Janzen and Paukstis 1991, M. B. Thompson pers. comm). The population of captively incubated and reared skinks used in this study will be released onto Korapuki Island, as part of the restoration goals for the island. To assess the long-term effects of constant, captive incubation regime on this species, it is important that this population is monitored after release.

2.5 References

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CHAPTER THREE
Incubation regime affects performance of the egg-laying skink, *Oligosoma suteri*

3.1 Introduction

Biological fitness is the ability of an organism to transfer its genes to the next generation (Hale *et al.* 1995). Ideally, studies of biological fitness should estimate the contribution of individuals, genotypes and phenotypes to the gene pool for each generation. However, such data are often impossible to obtain. Instead, performance correlates of phenotypic traits, such as size and growth, are frequently used to infer overall fitness of individuals, by their presumed influence on an organism's ability to survive to maturity and thus contribute to the gene pool (Russell 1998). In oviparous reptile species, phenotypic traits such as hatchling size, survival, growth and locomotor fitness can be influenced by incubation conditions (e.g. Brana and Ji 2000, Van Damme *et al.* 1992).

Large hatchling lizards (Ferguson and Fox 1984, Ferguson and Joanen 1982, Fox 1978), snakes (Jayne and Bennett 1990), and turtles (Alho *et al.* 1985, Janzen 1993) survive the neonatal period better than small hatchlings in the same cohort. Large hatchlings are assumed to be superior to small hatchlings in competition for food and in locomotor performance, which in turn allow larger animals to grow faster and reach maturity sooner (Froese and Burghadt 1974, Jayne and Bennett 1990, Miller *et al.* 1987).

Size differences in hatchlings can result from variation in initial egg size, egg quality, incubation temperature, hydric conditions or combinations of these factors (Crump 1984, Packard and Phillips 1994). Usually, hatchlings are larger at optimal incubation temperatures and wetter water potentials. For example, *Crocodylus niloticus* hatchlings are significantly longer from eggs incubated at 31°C than at 28°C or 34°C (Hutton 1987), and painted turtles (*Chrysemys picta*) are larger when hatched from eggs

incubated at wet (-150 kPa) rather than dry (-1500 kPa) water potentials (Packard *et al.* 1991). Incubation temperature also has long-term effects on post-hatching growth, behaviour and locomotor performance in reptiles (Burger 1989, Joanen *et al.* 1987). For example, individuals of the skink *Bassiana duperreyi* incubated in warm conditions (27°C ± 4°C) are faster than cool incubated siblings (20°C ± 4°C) to 20 weeks of age (Elphick and Shine 1998).

As the only endemic oviparous lizard in New Zealand (Towns 1975a, Thoresen 1967, Whitaker 1968), *Oligosoma suteri* is the only species whose eggs can be subjected to widely fluctuating incubation conditions. The aim of this chapter is to search for differences in fitness correlates among different incubation groups of *O. suteri*, focusing on the effects of incubation temperature and water potential regimes on hatchling size, growth, survival, and sprint speed.

3.2 Methods

Study area and egg collection methods are described in detail in Chapter 2, sections 2.2.1, 2.2.2 and 2.2.3.

3.2.1 Incubation of eggs

A brief summary of egg incubation is presented here. For more detail refer to Chapter 2, section 2.2.4.

One hundred and seventy four *O. suteri* eggs were randomly assigned among three incubation temperatures (18°C, 22°C and 26°C) and two water potentials (-120 kPa and -270 kPa). Eggs were initially placed in separate plastic cups, but when some eggs began to dehydrate, the eggs were reassigned to 2 L plastic containers. Water potential of the medium grade vermiculite used as an incubation medium was achieved by mixing 100 g of vermiculite with 96.4 ml (-120 kPa) or 32.6 ml (-270 kPa) of water. Distilled water was added every seven days to compensate for small losses from the container and uptake by the eggs. A Q_{10} for incubation period was calculated from incubation periods at 22°C and 26°C (2.46) and used to predict the incubation period for 18°C eggs (105 days). When the 18°C eggs had not hatched 30 days after the predicted day of hatching, they were randomly assigned to either 18°C or 22°C for the remainder of incubation.

3.2.2 Maintenance of juveniles

Immediately following hatching, juveniles were uniquely toe-clipped to allow for identification (Appendices I and IIa), weighed to 1 mg (on a Sartørius top loading balance), and total-length (TL) and snout-vent length (SVL) measured. Length was measured to 0.5 mm with a 150 mm clear plastic ruler. Juveniles were weighed each week, and measured monthly for four months.

Juveniles were housed in identical conditions in groups of three in transparent plastic boxes (New Ocean™ Plastic No. 838, 215 x 330 x 110 mm, 7 L), with a square of wire mesh (165 x 120 mm) on the lid for airflow. Boxes contained moist, non-fertilised potting mix in the bottom (at least 20 mm deep), pieces of pine (*Pinus radiata*) bark for shelter, and water provided *ad libitum* in a round plastic bowl (30 mm deep, 70 mm

diameter). Individuals incubated at 18°C were given shallower (10 mm deep, 110 mm diameter) water dishes due to their inability to exit the deeper ones. To allow for behavioural thermoregulation, skinks had a choice of temperatures, ranging from room temperature (>14.5°C) to 30°C (provided by a strip heater along the back of each box). The boxes were stacked on metal shelves with simulated natural lighting (Duro-test® True-lite® power twist) suspended from the ceiling on a 12L:12D light cycle (lights on at 0600 h). Boxes were moved weekly to allow all juveniles access to the light. They were fed three times a week with nymph crickets (*Telleogryllus commodus*) or vestigial winged fruit flies (*Drosophila melanogaster*), which were periodically laced with vitamin supplement powder (at least once a week). As juveniles grew, slaters (*Porcellio spp.*) and houseflies (*Musca domestica*) were also added to their diet.

3.2.3 Locomotor performance

A wooden race track (70 mm x 1.5 m), with 5 paired infrared lights in slots (0.25 m apart and 4 mm high) over 1 m, was used to test sprint speed. The five infrared lights transmitted and received an infrared beam horizontally across the track. When the first infrared beam was interrupted, four counters, with a pulse rate of 1024 s^{-1}, started on a digital display. The interruption of each successive infrared beam stopped one of the timers (modified from Huey *et al.* 1981). A horsehair paintbrush was used to encourage running in juveniles by gently touching their tails.

Juvenile sprint speed was tested at one and four months of age with the exception of fifteen 22°C and 26°C juveniles, which, due to electronic problems with the race track, were sprinted at six weeks. Each age group was sprinted on three consecutive days, with a different ambient temperature (18°C, 22°C and 26°C ± 0.5°C) each day. Individuals were sprinted three times each day at one temperature and given at least 15 minutes rest between each test. The order of ambient temperatures was randomised to control for habituation effects. The optimal temperature for *O. suteri* is unknown, and similar experiments on lizards use temperatures in this range (e.g. Elphick and Shine 1998). Juveniles were kept separately in 2 L plastic containers and left undisturbed for at least 30 minutes prior to the sprint test to acclimate to the ambient temperature. The race track was similarly warmed or cooled to the required temperature.
Before the sprint speed test, juveniles were weighed and measured (SVL and TL), each individual also had foot length (FL) and leg length (LL) measured to 0.25 mm. The

right rear foot was measured on the underside from the heel to the base of the web between the third and fourth digits. The right rear leg was measured along the lateral side from the groin to the ankle. The right and left sides of the animal were viewed from the dorsal surface.

3.2.4 Data analysis

Data were analysed using the statistics packages SPSS and S-plus. All data were tested for normality using exploratory analyses on the statistics program SPSS, and statistical significance was assumed at $p < 0.05$. Data are expressed as mean ± 1 standard error (SE) unless otherwise stated.

The effect of changing from cups to boxes during incubation versus only incubating in boxes was tested by a MANOVA, with a nested water potential and temperature design on SYSTAT. Clutch effects were not investigated as all clutches had five eggs or fewer, too small for powerful statistical tests to be carried out (pers. comm. Edith Hodgen). Thus, the effect of maternal size (SVL) and initial egg mass were used as covariates in analyses as substitutes to allow for any intra-clutch variation. Two very small individuals (outliers) were removed before analysis of hatchling size and growth, which did not alter the results, but did increase the power of the tests.

3.2.4.1 Hatchling size

Only those individuals that hatched without assistance (versus those cut from eggs) were included in hatchling size analyses. Fully developed embryos that failed to hatch unaided suffered from *rigor mortis* and were too bent to measure accurately, nor was it known whether individuals cut from eggs had reached their maximum size. The effect of incubation regime on hatchling size was assessed using a multivariate general linear model (GLM) on SPSS, with incubation treatments as independent factors, the dependent factors of hatchling size (including SVL, vent-tail length (VTL - obtained by subtracting SVL from TL), TL and Wt), and sex and initial egg mass as covariates.

3.2.4.2 Survival

The effect of incubation regime and initial egg mass on survival to four months of age was assessed using logistic regression models on S-Plus, with the independent factors of incubation treatments (incubation temperature and water potential as categorical data), initial egg mass and sex, and the dependent variable of probability of survival

(1 = survived, 0 = died). Akaike information criteria were used to determine the best-fit model and which independent factors did not contribute to survival (McCallum 2000). Chi-squared (χ^2) tests were used to compare models. As 95% confidence intervals can take the upper limit of probability above one and probability of survival cannot be greater than one (1 = survived), 95% profile likelihood intervals were used.

3.2.4.3 Juvenile growth

Growth to four months of age was assessed using a repeated measures GLM on SPSS, with between-subject factors of incubation temperature and water potential, within-subject factors of month including size measures (SVL, VTL, TL and mass), and covariates of sex, hatchling size and initial egg mass. Individuals that did not survive to four months of age were excluded from the analysis.

Change in condition from hatching to four months was assessed using ANOVA on SPSS. Condition was measured by an index $\Delta Ci = \Delta mass/\Delta TL$ (modified from Ussher 1999).

3.2.4.4 Locomotor performance

Maximum sprint speed over 0.25 m was used instead of the full 1 m as many individuals paused at least once within 1 m. Average sprint speed was not used as an estimate of maximum sprint capacity because only burst speed was correlated with significant survivorship in garter snakes, *Thamnophis sirtalis fitchi* (Jayne and Bennett 1990). Sprint speed was assessed using a repeated measures GLM on SPSS, with between-subject factors of incubation temperature and water potential, within-subject factors of month and sprint temperature, and covariates of sex, mother size, initial egg mass and most juvenile size measures (including SVL, TL, FL, LL, mass and condition index). Vent-tail length was not used as it was not independent data, that is, it was derived from SVL and TL. Any individuals that did not survive to four months of age were excluded from the analysis. Three outliers were removed before analysis as these individuals either did not sprint 0.25 m without stopping, or leaped over the infrared beam, giving no reading for that sector.

3.3 Results

There were no significant differences in hatchling size, survival and growth for those individuals incubated only at 18°C and those that had incubation temperature raised to 22°C near the end of incubation. Therefore, all parameters for these individuals were combined for further analyses. Similarly, there was no significant influence of changing from cups to boxes and no box affect, which allowed for removal of these parameters for the remainder of the analyses. Results from all statistical tests, significant and otherwise, are presented in Appendix IIIb.

3.3.1 Hatchling size, survival and growth

Sex of the hatchlings did not influence their size within treatment groups ($F = 0.338$, $df = 4$, $p = 0.852$), and was thus removed from further hatchling size analysis. Hatchling size was affected by temperature ($F = 12.248$, $df = 6$, $p < 0.001$), water potential ($F = 4.597$, $df = 3$, $p = 0.005$) and initial egg mass ($F = 30.218$, $df = 3$, $p < 0.001$), but not any combination of these three factors. Hatchling SVL (Figure 3.1) and mass (Figure 3.2) were largest at 22°C. Hatchling VTL (Figure 3.3) and TL (Figure 3.4) were longest at the two warmer (22°C and 26°C) temperatures. The effect of water potential on these measures was most pronounced at 26°C, with very long tails occurring for individuals incubated at -120 kPa. Larger eggs produced larger hatchlings.

Post-hatching juvenile survival was influenced by incubation temperature ($\chi^2 = 43.023$, $df = 132,2$, $p < 0.001$, Table 3.1), but not sex ($\chi^2 = 0.475$, $df = 133, 1$, $p = 0.491$), water potential ($\chi^2 = 0.001$, $df = 133,1$, $p = 0.970$), initial egg mass ($\chi^2 = 0.622$, $df = 133,1$, $p = 0.431$), or any combination of incubation temperature with these 3 factors. The highest probability of surviving to four months occurred at 22°C and 26°C (Figure 3.5).

Growth to four months was influenced only by incubation temperature ($F = 5.137$, $df = 18$, $p < 0.001$); all other variables (water potential, sex, initial egg mass and hatchling size measures) had no effect. Individuals incubated at 22°C and 26°C grew faster, attaining a larger size in all size measures than 18°C individuals.

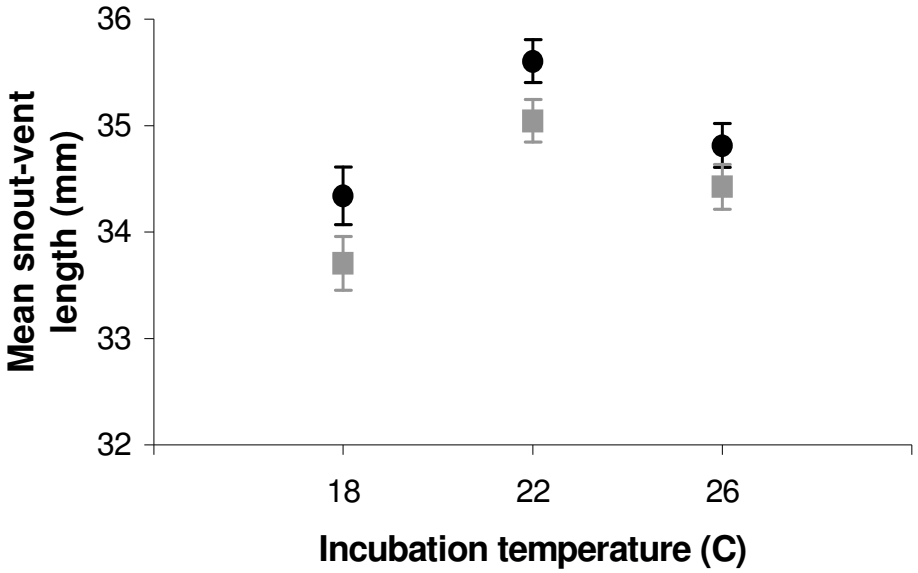

Figure 3.1 Mean snout-vent length (mm) ± SE of hatchlings for each incubation temperature (°C) and water potential (● = -120 kPa, ■ = -270 kPa). Note that 18°C includes individuals that completed the last part of incubation at either 18°C or 22°C.

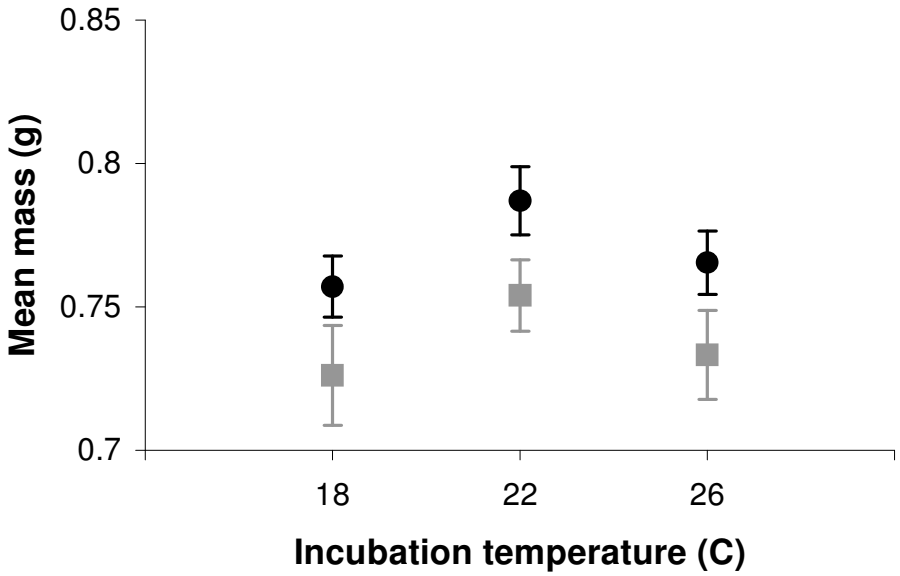

Figure 3.2 Mean mass (g) ± SE of hatchlings for each incubation temperature (°C) and water potential (● = -120 kPa, ■ = -270 kPa). Note that 18°C includes individuals that completed the last part of incubation at either 18°C or 22°C.

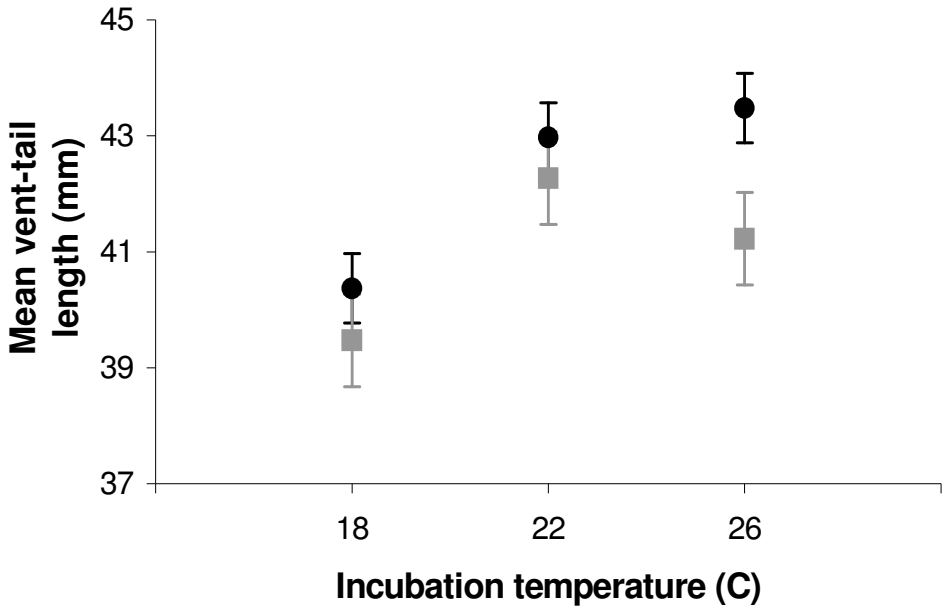

Figure 3.3 Mean vent-tail length (mm) ± SE of hatchlings for each incubation temperature (°C) and water potential (● = -120 kPa, ■ = -270 kPa). Note that 18°C includes individuals that completed the last part of incubation at either 18°C or 22°C.

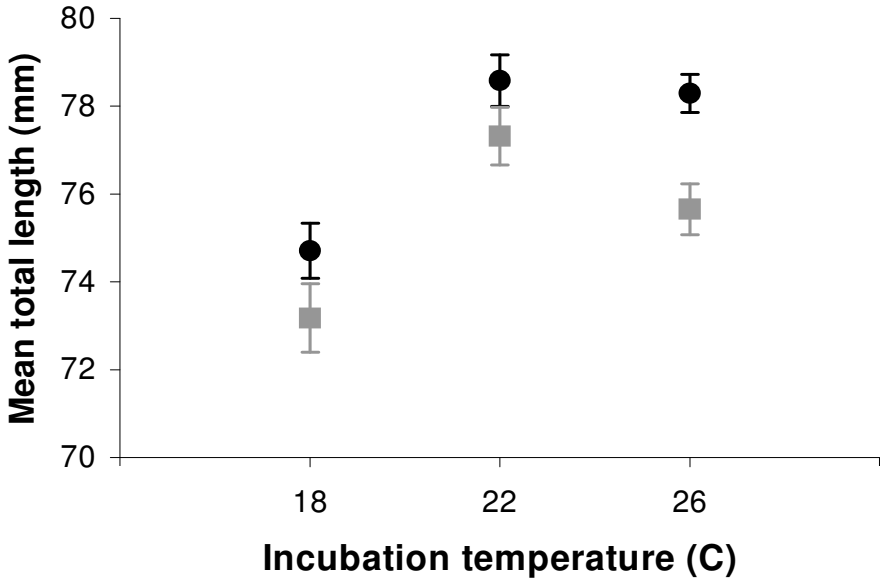

Figure 3.4 Mean total length (mm) ± SE of hatchlings for each incubation temperature (°C) and water potential (● = -120 kPa, ■ = -270 kPa). Note that 18°C includes individuals that completed the last part of incubation at either 18°C or 22°C.

Table 3.1 Hatchling survival amongst incubation temperatures (T = temperature).

T (°C)	No. Hatchlings	No. Died				No. alive at 4 months (%)
		Month 1	Month 2	Month 3	Month 4	
18	37	12	1	3	0	21 (57%)
22	50	0	0	0	0	50 (100%)
26	49	1	0	0	0	48 (98%)

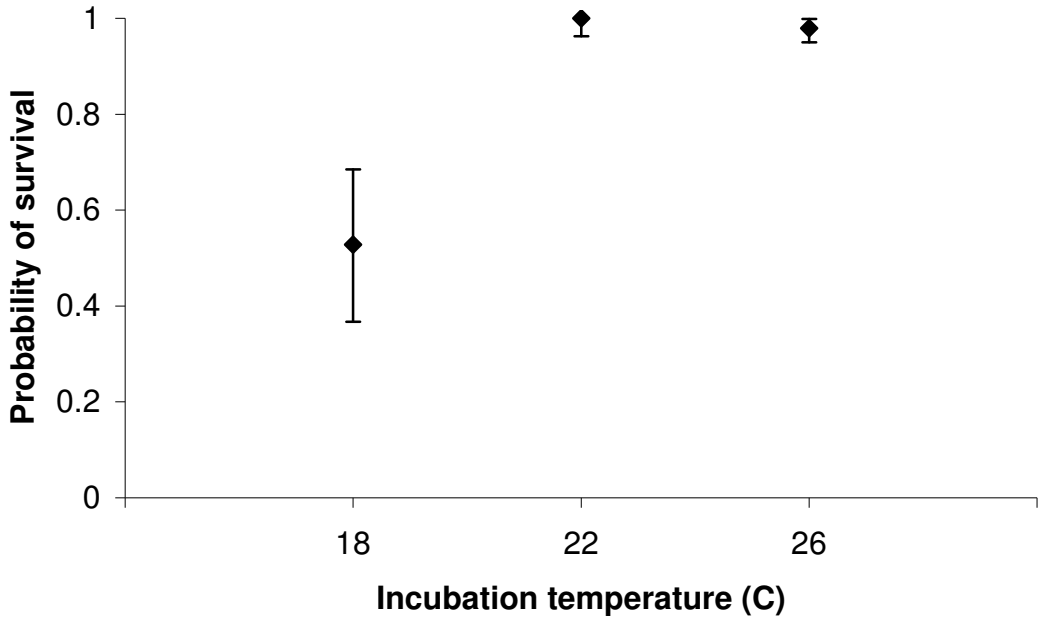

Figure 3.5 Probability of surviving at each incubation temperature ± 95% profile likelihood intervals. Note that 18°C includes individuals that completed the last part of incubation at either 18°C or 22°C.

Change in condition index from hatching to four months was influenced by incubation temperature ($F = 51.913$, $df = 2$, $p < 0.001$), but not water potential ($F = 1.501$, $df = 1$, $p = 0.223$), initial egg mass ($F = 0.777$, $df = 1$, $p = 0.380$), or a combination of the two incubation treatments. Condition index was not significantly different between the three incubation temperatures at hatching, but by four months the individuals incubated at 22°C and 26°C had a greater condition index than the 18°C individuals (Figure 3.6), that is, they weighed more per unit length. However, at four months individuals incubated at 26°C and -270 kPa weighed less per unit length than those incubated at 26°C and -120 kPa.

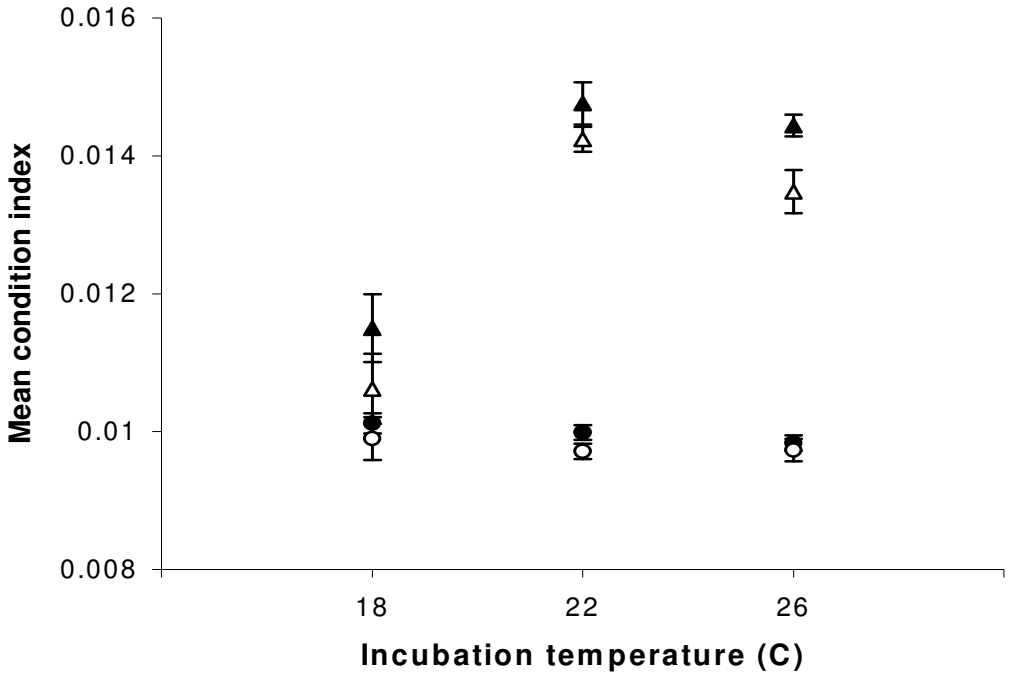

Figure 3.6 Mean condition index ± SE for each incubation temperature and water potential at hatching and four months (● = 0 months at -120 kPa, ○ = 0 months at -270 kPa, ▲ = 4 months at –120 kPa, Δ = four months at –270 kPa). Note that 18°C includes individuals that completed the last part of incubation at either 18°C or 22°C.

3.3.2 Locomotor performance

Sprint speed was influenced by incubation temperature ($F = 9.348$, $df = 2$, $p < 0.001$) and condition index ($F = 9.348$, $df = 1$, $p = 0.003$), but not by water potential, sex, initial egg mass, size measurements (snout-vent length, total length, mass, leg length or foot length), or maternal SVL, although initial egg mass had an effect when both month and sprint temperature were combined ($F = 6.968$, $df = 2$, $p = 0.002$). All individuals had faster sprint speeds at warmer ambient temperatures. For all ambient temperatures at one month, individuals from the warmer incubation temperatures (22°C and 26°C) were faster, with no significant difference between the two (Figure 3.7). The same trend was apparent for four month sprint speeds, with no difference between sprint speed at one and four months for 22°C and 26°C incubated individuals, with the exception of 26°C (-270 kPa) individuals which were slower at four months at the ambient temperature of 26°C. The 18°C individuals were significantly slower over all ambient temperatures at four months (Figure 3.7).

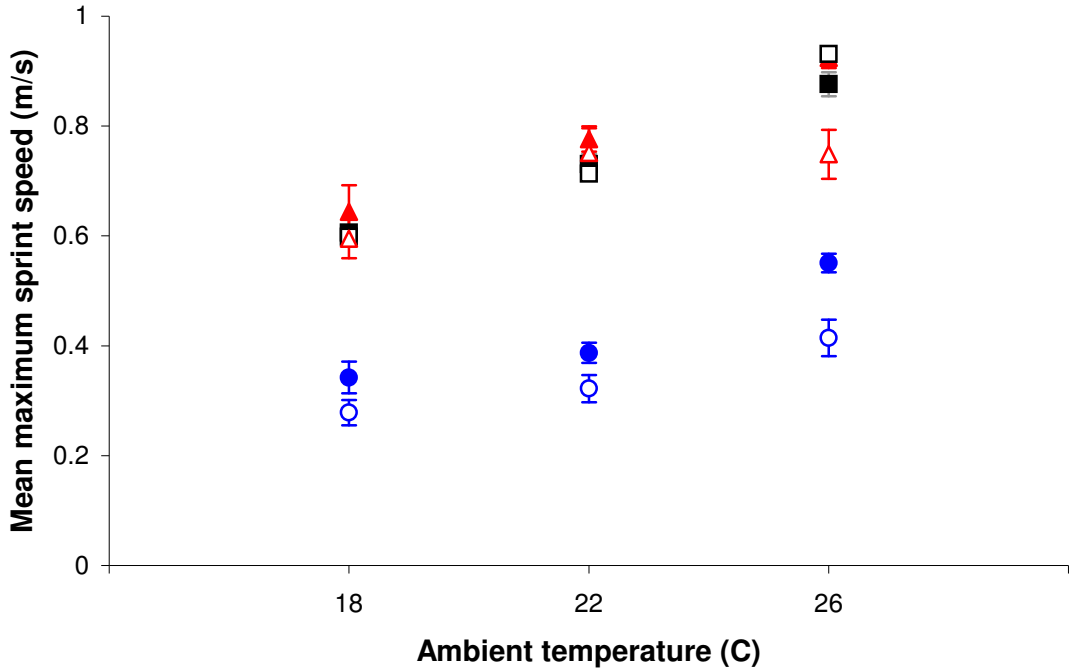

Figure 3.7 Mean maximum sprint speeds (ms^{-1} ± SE) at each ambient temperature for individuals from each incubation temperature at one and four months (● = 1 month and 18°C incubation temperature, ○ = 4 months and 18°C incubation temperature, ■ = 1 month and 22°C incubation temperature, □ = 4 months and 22°C incubation temperature, ▲ = 1 month and 26°C incubation temperature, Δ = 4 months and 26°C incubation temperature). Note that 18°C includes individuals that completed the last part of incubation at either 18°C or 22°C.

3.4 Discussion

3.4.1 Hatchling size, survival and growth

Large hatchlings are thought to be superior to small hatchlings due to improved competitive ability, as well as greater locomotor performance and thus success at evading predators (Froese and Burghadt 1974, Morris *et al.* 1983, Sinervo and Huey 1990). An increase in hatchling size at an optimal (increased survival) temperature is a common phenomenon in oviparous reptiles (e.g. Choo and Chou 1987, Jensen 1982, Lang *et al.* 1989, Marcovaldi *et al.* 1997, Van Damme *et al.* 1992, Ynetma 1978). For most measurements, hatchlings of *O. suteri* were larger at a constant incubation temperature of 22°C, although tail length of 26°C individuals was significantly longer at -120 kPa. A similar pattern occurs in some other squamates where hatchlings are larger from moderate incubation temperatures than from low or high incubation temperatures. For example, hatchlings of the agamid lizard *Ctenophorus ornatus* (Harlow 2000) and the bull snake *Pituophis melanoleucus* (Gutzke and Packard 1987) are larger from intermediate incubation temperatures. The bull snake also suffers from physiological abnormalities and low hatching success at high incubation temperatures close to the upper lethal limit (Gutzke and Packard 1987).

Influence of water potential on hatchling size has mostly been studied in turtles, where larger hatchlings are produced at wetter water potentials, such as in the snapping turtle, *Chelydra serpentina* (Morris *et al.* 1983). This pattern is also common in squamates, as eggs of oviparous squamate reptiles usually have thin, flexible shells with little resistance to water movement from the surrounding environment (Packard *et al.* 1982). In *O. suteri* hatchling size was positively correlated with water potential.

Hatchling survival probability was significantly greater at the two warmer (22°C and 26°C) incubation temperatures. Incubation temperature also influences survival probability of other oviparous reptile neonates, such as in desert tortoises (*Gopherus agassiz*), where highest survival to 277 days occurs at optimal temperatures (28°C, 29°C and 31°C) compared with sub-optimal temperatures of 25°C and 27°C (Lewis-Winokur and Winokur 1995).

Many studies of reptiles show a relationship between survival and body size. For example, in wild populations of the lizard *Uta stansburiana*, a greater proportion of large than small individuals survive to breeding age (Ferguson and Fox 1984). However, there are exceptions, such as in a population of snapping turtles (*Chelydra serpentina*), where a seven year study failed to show any correlation between large size and higher survival (Congdon *et al.* 1999). For relict mainland populations of *O. suteri*, which experience mammalian predators and human interference, faster growth rates and larger size are likely to be of an advantage to individuals, allowing quicker attainment of maturity and potentially earlier reproduction.

In *O. suteri*, growth to four months was faster in neonates from the two warmer incubation temperatures than those from 18°C. Effects of incubation regime on growth are also common in other reptiles, such as hatchling Cuban rock iguanas (*Cyclura nubila*), which grow faster in their first year when incubated at high (31°C) incubation temperatures (Alberts *et al.* 1997).

The condition indices of *O. suteri* hatchlings from all incubation regimes were similar, probably due to individuals receiving approximately the same amount of nutrients from eggs. Eggs were all of similar mass regardless of maternal size (Chapter 2, section 2.3.1). At four months, 22°C and 26°C incubated individuals had a significantly higher condition index, which is consistent with casual observations that 18°C individuals had reduced ability to catch food.

There was no difference in hatchling size and growth between the sexes, which was expected as there is no external secondary sexual dimorphism in *O. suteri*. Ventral colouration differs between sexes during the breeding season, although this is not a definitive sex determining character (Towns 1975b). The genus *Oligosoma* typically shows little sexual dimorphism, with individuals often difficult to sex without dissection (Barwick 1959).

3.4.2 Locomotor performance

Greater locomotor performance, such as faster sprint speed, may allow individuals to evade predators more successfully and increase ability to catch food, which can in turn increase growth rate, and thus size at maturity and potentially reproductive success (Downes and Shine 1999, Froese and Burghadt 1974, Jayne and Bennett 1990). Faster speed has been correlated with both incubation regime and individual size in many reptiles (e.g. Elphick and Shine 1998, Macrini and Irschick 1998, Miller *et al.* 1987, Sinervo 1990). Size does not influence sprint speed in juvenile *O. suteri*, but incubation temperature does, with those individuals incubated at 22°C and 26°C sprinting faster than 18°C individuals. Sprint speed is also positively correlated with condition index. No individuals sprinted faster at four months than one month; moreover, a slower burst speed (at four months compared with one month) was obtained for individuals incubated at 18°C and 26°C (-270 kPa). This is the first study to report a decrease in sprint speed as individuals get older. It is worth noting that the smaller hatchling size of 26°C incubated individuals and slower sprint speed at warmer ambient temperatures indicate that 26°C may be close to, but not at, the upper lethal limit.

3.4.3 Implications

Like all reptiles, *O. suteri* physiology is influenced by incubation temperature. Current global warming predictions estimate an increase in mean global temperature of between 1.4°C and 5.8°C and an increase in sea level of between 9 mm and 88 mm by 2100 (IPCC 2001). Habitat preference and restricted distribution of *O. suteri* make this species particularly vulnerable to global climate change and sea level rises. However, sea level rise may prove to be detrimental only if island sanctuaries are completely submerged, whereas data on natural nest temperatures are needed before effects of increased global temperatures can be ascertained.

Potentially, the mainland populations of *O. suteri* could extend their range southward if global temperatures increase, but suitable boulder beach habitats are not found in a continuum. Also, due to mammalian predators and human interference, most populations of this skink occur on offshore islands (Towns and Daugherty 1994), which, when coupled with sea level rise, indicates that they will have limited opportunity to disperse towards suitable, cooler habitats.

3.4.4 Conclusion

The data presented in this chapter are consistent with previous studies that have demonstrated differences in phenotypic traits of hatchling reptiles in response to incubation conditions (e.g. Brana and Ji 2000, Miller *et al.* 1987, Shine *et al.* 1997, Steyermark and Spotila 2001). However, the data are based entirely on the first four months of life, and *O. suteri* are long lived (at least 12 years on offshore islands, Towns and Ferreira 2001). Their phenotypic responses to incubation conditions may alter with age, although this is very unlikely in the 18°C incubated individuals. Nonetheless, other studies have suggested that incubation induced phenotypic changes may persist for long periods of time (Andrews *et al.* 2000, Elphick and Shine 1998, Joanen *et al.* 1987, Shine *et al.* 1995), although this is by no means direct evidence of a link with biological fitness. The population of captively incubated and reared skinks used in this study will be released onto Korapuki Island, as part of the restoration goals for the island. This provides an excellent opportunity to assess the long-term effects of constant, captive incubation regime on phenotypic traits and ultimately biological fitness of *O. suteri*.

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CHAPTER FOUR

Summary and recommendations

This thesis contributes to the understanding of the reproductive biology of *Oligosoma suteri* by demonstrating the effects of incubation regime on various ecological factors, in particular, how incubation temperatures and water potentials affect incubation period, hatchling morphology, sex, embryonic and juvenile survival, hatchling growth and juvenile locomotor performance.

Oligosoma suteri eggs (n = 174) were randomly distributed among three different constant incubation temperatures and two water potentials. Incubation period, embryonic development, hatchling size, growth, survival and sprint speed were affected by incubation regime, with the greatest influence accredited to temperature. Sex of individuals was not influenced by incubation regime, and therefore *O. suteri* do not have temperature-dependent sex determination. The lower lethal limit is close to or at 18°C, and constant 26°C incubation may be close to the upper lethal limit.

4.1 Summary of results

- At oviposition embryos are at stage 32 according to the staging series for *Lacerta vivipara*, which is two stages later than for most other oviparous reptiles.
- Maternal size (SVL) and clutch size are positively correlated as in other reptile species.
- Incubation period is longer at cooler temperatures, with no influence attributed to an increase in temperature in the final 10 to 29 days of incubation.
- Change in egg mass during incubation is positively correlated with incubation temperature, water potential and initial egg mass.
- Hatching success is greatest at warmer incubation temperatures (22°C and 26°C).
- Hatchlings from constant 18°C incubation suffer from physical abnormalities, similar to other squamate reptiles incubated at or near the lower lethal limit.

- Sex ratio is close to 50:50 for all incubation treatments, so sex is probably genetically determined.
- Hatchling size is larger, in most measurements, at constant 22°C incubation and -120 kPa. Growth is greatest in hatchlings incubated at the two warmer incubation temperatures.
- Condition index (mass per unit length) is similar for all hatchlings, but only 22°C and 26°C individuals increased their condition after four months.
- Survival to four months is higher in individuals hatched from the two warmer incubation temperatures; only 57% of hatched individuals survive from 18°C incubation.
- Sprint speed is positively correlated with incubation temperature and condition index but not influenced by size or age of individuals. Furthermore, individuals incubated at 18°C or 26°C (-270 kPa) had slower sprint speeds at four months than at one month.

4.2 Recommendations

Future captive rearing of *O. suteri* should use constant 22 °C and -120 kPa, ensuring larger hatchlings with higher survival probability and greater fitness measures to at least four months of age. Translocation of *O. suteri* populations should only be done within their current geographic range.

4.3 Future research

Based on the findings from this thesis, I suggest four future avenues of research: a) determine natural nest temperatures of *O. suteri* and analyse how increases in global temperatures will influence *O. suteri*, b) study the long-term effects of constant, captive incubation regimes on this species by monitoring the population after release, c) test fitness measures of natural populations and compare with the captive incubated individuals, and d) determine whether *O. suteri* has heteromorphic sex chromosomes.

APPENDIX I

Toe-clip information

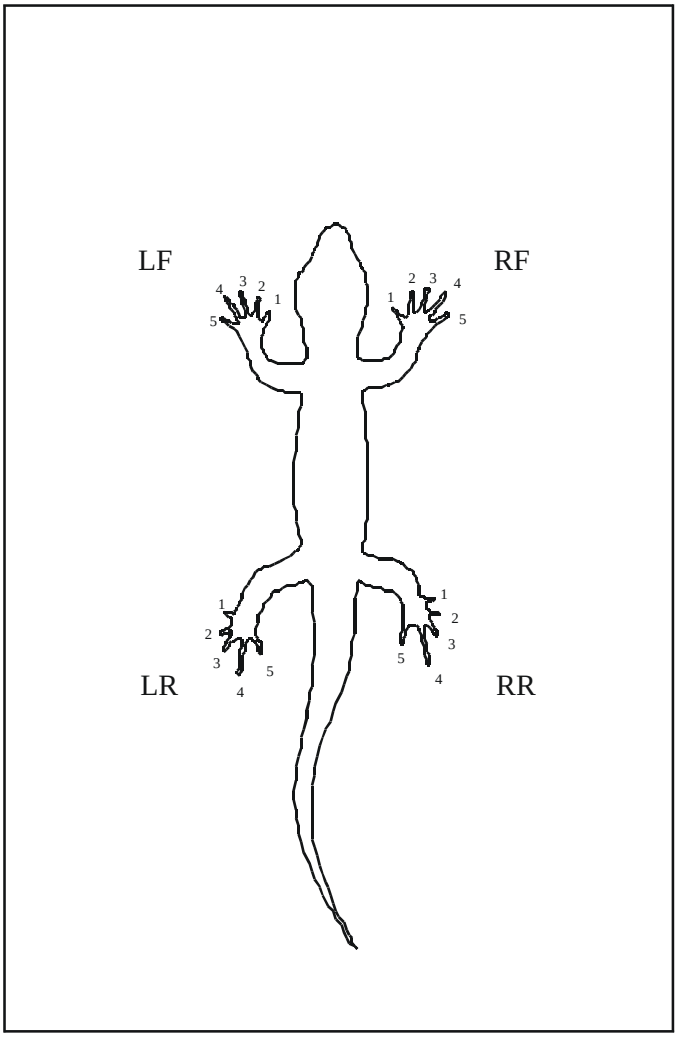

Figure I Dorsal view of skink; numbers indicate toe number. (LF = left front, RF = right front, LR = left rear, RR = right rear).

A) Green Island Female *Oligosoma suteri*, December 1999

Table Ia Toe-clips used on female *O. suteri* taken from Green Island in December 1999. A list is present at the end of the table describing abbreviations used.

No.M	T-C	FT	Natural	SVL	VTL	R	Mass	Date Laid	No.E
1	5555	5475	-	103	74	32	16.425	31-Dec-99	5
2	5554	5476	LR	99	56	34	12.465	06-Jan-00	4
3	5553	5480	-	92	78	30	11.08	13-Jan-00	3
4	5552	5482	-	94	53	40	11.236	21-Dec-99	3
5	5551	5510	RR	97	68	36(15)	13.085	29-Dec-99	4
6	5545	5481	-	93	67	28	10.989	03-Jan-00	4
7	5544	5477	RR	96	34	24	10.13	16-Jan-00	2
8	5543	5483	-	97	79	22	13.698	06-Jan-00	4
9	5542	5484	-	98	82	36	11.778	03-Jan-00	3
10	5535	5486	-	99	59	41	13.03	02-Jan-00	2
11	5534	5488	-	91	89	6	10.74	02-Jan-00	3
12	5533	5485	RR	103	88	44	14.428	06-Jan-00	5
13	5532	5490	-	98	55	39	11.587	02-Jan-00	4
14	5531	5491	LR, RR	96	83	39	12.726	08-Jan-00	4
15	5525	5492	-	98	72	51	-	-	-
16	5524	5489	RR	95	31	19	10.039	05-Jan-00	3
17	5523	5494	-	97	87	33	12.629	06-Jan-00	3
18	5522	5496	-	96	71	48	11.76	17-Jan-00	4
19	5455	5497	-	99	74	37	13.694	02-Jan-00	3
20	545(45)	5500	RF, RR(4)	90	92	18	10.434	29-Dec-99	4
21	5454	5498	RR	102	48	35	13.42	15-Jan-00	4
22	5453	5499	-	101	74	49	13.79	30-Dec-99	4
23	5452	5501	-	90	85	23	11.728	31-Dec-99	3
24	5445	5495	LR	102	65	46	-	-	-
25	5444	5502	-	99	105	12	12.89	03-Jan-00	4
26	5443	5503	-	91	68	50	10.356	29-Dec-99	3
27	5442	5504	-	95	76	24	11.042	29-Dec-99	5
28	5435	5505	-	94	53	41	10.541	02-Jan-00	3
29	5434	5506	-	97	79	28	12.518	01-Jan-00	4
30	5433	5507	LF	100	41	27	10.946	03-Jan-00	4
31	5432	5508	-	92	41	37	10.064	24-Dec-99	3
32	5425	5509	-	95	55	48	11.56	06-Jan-00	3
33	5424	5513	-	101	93	c	13.43	21-Jan-00	4
34	5423	5514	-	93	43	28(7)	10.193	02-Jan-00	3
35	5422	5515	-	92	80	37	12.892	27-Dec-99	3
36	5355	5487	RF	103	75	45	15.789	31-Dec-99	4
37	5354	5493	RF	95	56	39	11.006	03-Jan-00	2
38	5353	5517	-	96	37	0	12.396	08-Jan-00	4
39	5352	5518	-	93	64	44	10.475	28-Dec-99	4
40	5345	5516	LR	93	77	22	12.459	29-Dec-99	3
41	5344	5520	-	95	64	33	12.282	03-Jan-00	4
42	5343	5521	-	94	64	39	10.952	23-Dec-99	3
43	5342	5522	LF	96	40	5	11.42	12-Jan-00	4
44	5335	5519	RR	95	59	32	10.706	06-Jan-00	3
45	5334	5524	-	99	73	47	15.341	04-Jan-00	4

No.M	T-C	FT	Natural	SVL	VTL	R	Mass	Date Laid	No.E
46	5333	5525	-	103	67	57	14.897	06-Jan-00	5
47	5332	5526	-	95	66	55	12.462	05-Jan-00	4
48	5325	5527	RR	97	56	41	13.16	17-Jan-00	3
49	5255	5528	RR	102	65	48	13.543	06-Jan-00	4
50	5254	5511	LF, RF, RR	101	45	26	14.398	04-Jan-00	4
51	4455	5523	RF	96	47	32	11.616	08-Jan-00	4
52	3555	5478	LF	97	67	46	12.19	03-Jan-00	4
53	3554	5529	LF	97	57	41	12.05	14-Jan-00	3
54	2555	5512	LF	92	78	32	11.319	09-Jan-00	4
55	1555	5479	LF	100	76	42	14.684	07-Jan-00	4
56	foot_ _ _	-	LF	97	64	31	12.257	31-Dec-99	4
57	_foot_ _	-	RF	90	95	10	10.937	6-Jan-00	4
58	_2_foot	-	RF, RR	97	57	46	11.81	14-Jan-00	3
59	_ _ 3(35)	-	LR, RR	103	62	46	13.87	19-Jan-00	4
60	_ _ _(345)	-	RR	95	60	49	12.524	8-Jan-00	4

Abbreviations used

No.M	=	an arbitrary number assigned to female.
T-C	=	toe clip - read LF, RF, LR, RR viewed from dorsal surface (Figure I), LF = left front, RF = right front, LR = left rear, RR = right rear.
Natural	=	toes, or feet, that were lost naturally and thus not toe-clipped.
SVL	=	the length (mm) from snout to cloacal opening (vent).
VTL	=	the length (mm) of the tail in its entirety from vent to tip (i.e. including any regeneration).
R	=	the length (mm) of tail regeneration. Numbers in brackets indicate that there was more than one regeneration area; 'c' indicates that tail was complete.
Wt	=	the mass (g) of females immediately after oviposition.
Date Laid	=	the date the female oviposited.
No.E	=	the number of eggs the female laid.
Dash (-)	=	not applicable.

The detached toes were assigned frozen tissue (FT) numbers and stored in Nuncs™ filled with 95% ethanol while on Green Island and put into an -80 °C freezer at VUW. The freshly clipped toes were dipped in Tricin™ powder (triple antibiotic powder: Bacitracin zinc, Neomysin sulphate and Polymyxin B sulphate) to prevent infection. Length measurements were obtained using a 300 mm clear plastic ruler and mass using a Sartørius top-loading balance.

B) Captive incubated and reared *O. suteri*

Table Ib Toe-clips and hatching data for juvenile *O. suteri* incubated at constant incubation temperatures. A list is present at the end of the table describing abbreviations used.

T-C	T	WP	SEX	SVL	TL	Wt	No.M	DateL	DateH	FT	Notes
5555	26	-120	F	32	74	0.679	40	29/12/99	9/2/00	5575	cod
5554	26	-120	M	35	79	0.822	31	24/12/99	14/2/00	5578	
5553	26	-120	F	36	81	0.835	4	21/12/99	15/2/00	5576	
5552	26	-270	M	29	63	0.416	35	2/1/00	15/2/00	5577	
5545	26	-270	F	34	76	0.689	13	2/1/00	23/2/00	5560	
5544	26	-270	M	33	76	0.826	42	3/1/00	17/2/00	5555/85	
5543	26	-120	M	31	73	0.799	-	18/2/00	18/2/00	5554/82	
5542	26	-120	F	38	84	0.859	-	18/2/00	18/2/00	5580	
5535	26	-270	F	33	69	0.733	56	31/12/99	18/2/00	5581	D26/07/00
5534	26	-120	F	34	73	0.638	20	29/12/99	19/2/00	5556	
5533	26	-120	M	34	81	0.701	20	19/2/00	19/2/00	5553	
5532	26	-270	F	34	74	0.683	27	29/12/99	19/2/00	5557	
5525	26	-120	F	35	79	0.777	22	30/12/99	21/2/00	5579	
5524	26	-270	F	34	72	0.634	45	4/1/00	21/2/00	5552	
5523	26	-120	M	36	79	0.782	19	2/1/00	21/2/00	5558	
5522	26	-120	F	35	80	0.794	9	3/1/00	23/2/00	5559	
5455	26	-120	M	34	79	0.781	34	2/1/00	23/2/00	5561	
5454	26	-120	F	33	75	0.71	32	6/1/00	23/2/00	5562	
5453	26	-120	M	35	76	0.765	16	5/1/00	24/2/00	5563	
5452	26	-120	F	34	80	0.767	63	3/1/00	24/2/00	5564	
5445	26	-270	F	35	77	0.747	47	5/1/00	25/2/00	5565	
5444	26	-120	F	35	79	0.777	2	6/1/00	25/2/00	5566	
5443	26	-120	M	36	81	0.837	41	29/12/99	25/2/00	5567	
5442	26	-120	M	35	79	0.748	8	6/1/00	25/2/00	5568	
5435	26	-120	F	35	78	0.76	46	6/1/00	26/2/00	5569	
5434	26	-270	M	35	76	0.752	49	6/1/00	26/2/00	5570	
5433	26	-270	F	33	75	0.671	43	12/1/00	2/3/00	5587	
5432	26	-270	M	34	73	0.67	12	6/1/00	26/2/00	5572	D13/3/00
5425	26	-120	M	36	80	0.763	50	4/1/00	26/2/00	5573	
5424	26	-120	M	36	79	0.81	51	8/1/00	27/2/00	5574	
5423	26	-270	M	35	78	0.765	-	27/2/00	27/2/00	5582	
5422	26	-270	F	36	77	0.741	60	8/1/00	28/2/00	5585	
5355	26	-120	F	34	77	0.746	54	9/1/00	3/3/00	5588	
5354	26	-270	F	34	76	0.678	3	13/1/00	3/3/00	5589	
5353	26	-270	M	34.5	76	0.743	53	14/1/00	4/3/00	5590	
5352	26	-120	M	34.5	77	0.745	53	14/1/00	6/3/00	5591	
5345	26	-120	M	35	77	0.681	58	14/1/00	6/3/00	5592	
5344	26	-120	M	35	78	0.779	21	15/1/00	6/3/00	5593	
5343	26	-120	F	36	80	0.858	48	17/1/00	7/3/00	5594	
5342	26	-270	M	34	75	0.641	18	17/1/00	7/3/00	5595	
5335	26	-270	M	36	77	0.762	57	6/1/00	26/2/00	5571	
5334	26	-270	F	36	82	0.909	7	16/1/00	9/3/00	5596	
5333	26	-270	M	35	78	0.806	59	19/1/00	10/3/00	5597	
5332	26	-270	M	35	75	0.813	59	19/1/00	10/3/00	5598	

T-C	T	WP	SEX	SVL	TL	Wt	No.M	DateL	DateH	FT	Notes
5325	26	-270	F	34	75	0.695	33	21/1/00	12/3/00	5599	
5324	26	-270	M	35	76	0.775	33	21/1/00	12/3/00	5600	
5323	22	-270	F	28	60	0.336	35	27/12/99	7/3/00	5601	
5322	22	-270	F	36	79	0.835	1	31/12/99	7/3/00	5602	
5255	22	-120	F	38	86	0.874	-	8/3/00	8/3/00	5603	
5254	22	-120	M	36	80	0.756	-	8/3/00	8/3/00	5604	
5253	22	-120	M	35	73	0.686	32	6/1/00	9/3/00	5605	cos
5252	22	-270	F	37	86	0.815	-	11/3/00	11/3/00	5606	
5245	22	-270	F	36	82	0.759	-	11/3/00	11/3/00	5607	
5244	22	-270	M	35	77	0.719	27	29/12/99	11/3/00	5608	
5243	22	-270	F	36	79	0.776	56	31/12/99	13/3/00	5609	
5242	22	-270	M	36	83	0.857	26	29/12/99	13/3/00	5610	
5235	22	-120	M	36	81	0.824	-	14/3/00	14/3/00	5611	
5234	22	-120	M	34	76	0.653	-	14/3/00	14/3/00	5612	
5233	22	-120	F	37	83	0.921	23	31/12/99	14/3/00	5613	
5232	22	-120	M	36	79	0.758	34	21/1/00	14/3/00	5614	
5225	22	-270	M	35	78	0.761	28	29/12/99	14/3/00	5615	
5224	22	-120	M	36	81	0.819	-	15/3/00	15/3/00	5616	
5223	22	-120	F	37	84	0.885	-	15/3/00	15/3/00	5617	
5222	22	-120	F	36	79	0.783	25	3/1/00	15/3/00	5618	
4555	26	-120	F	35	78	0.816	38	8/1/00	27/2/00	5584	
4554	26	-270	F	36	79	0.794	-	27/2/00	27/2/00	5583	
4553	26	-270	M	34	78	0.693	14	8/1/00	29/2/00	5586	
4552	22	-270	F	34	74	0.595	13	2/1/00	15/3/00	5619	
4544	22	-120	F	35	76	0.703	22	30/12/99	16/3/00	5620	
4543	22	-270	M	35	77	0.755	45	4/1/00	16/3/00	5621	
4542	22	-120	M	36	75	0.804	19	2/1/00	16/3/00	5622	
4535	22	-120	M	37	81	0.786	16	5/1/00	17/3/00	5623	
4534	22	-120	M	35	80	0.795	8	6/1/00	17/3/00	5624	
4533	22	-120	F	35	79	0.776	20	29/12/99	17/3/00	5625	
4532	22	-120	F	35	78	0.744	9	3/1/00	17/3/00	5626	D15/07/00
4525	22	-120	F	36	79	0.791	6	3/1/00	17/3/00	5627	
4524	22	-120	F	37	83	0.858	50	4/1/00	18/3/00	5628	
4523	22	-120	F	36.5	80	0.788	46	6/1/00	18/3/00	5629	
4522	22	-270	F	37	81	0.821	52	3/1/00	18/3/00	5630	
4455	22	-270	F	35.5	78	0.776	49	17/1/00	19/3/00	5631	
4454	22	-270	F	35	78	0.77	55	7/1/00	19/3/00	5632	
4453	22	-270	M	36	83	0.782	47	5/1/00	19/3/00	5633	
4452	22	-120	F	36	79	0.773	51	8/1/00	20/3/00	5634	
4444	22	-270	M	35	77	0.785	57	6/1/00	20/3/00	5635	
4443	22	-270	F	35	77	0.745	44	6/1/00	21/3/00	5636	
4442	22	-120	M	35	78	0.766	38	8/1/00	21/3/00	5637	
4435	22	-270	M	34	75	0.737	29	2/1/00	22/3/00	5638	
4434	22	-270	F	33	72	0.63	53	14/1/00	22/3/00	5639	
4433	22	-270	M	34	71	0.739	14	8/1/00	26/3/00	5642	
4432	22	-270	M	34	74	0.709	43	12/1/00	26/3/00	5643	
4425	22	-270	M	35.5	80	0.758	60	8/1/00	27/3/00	5644	
4424	22	-120	M	34	75	0.759	21	15/1/00	27/3/00	5645	
4423	22	-270	F	36	79	0.744	3	13/1/00	28/3/00	5646	
4422	22	-270	F	35	76	0.747	18	17/1/00	28/3/00	5647	

T-C	T	WP	SEX	SVL	TL	Wt	No.M	DateL	DateH	FT	Notes
4355	22	-120	M	34.5	79	0.814	54	9/1/00	29/3/00	5648	
4354	22	-120	M	35	77	0.82	58	14/1/00	30/3/00	5650	
4353	22	-120	M	35.5	80	0.828	48	5/1/00	30/3/00	5651	
4345	22	-270	F	35	78	0.802	59	19/1/00	2/4/00	5652	D31/01/01
4344	22	-270	M	34	75	0.746	33	21/1/00	2/4/00	5653	
4343	18	-270	M	34	75	0.74	5	29/12/99	4/5/00	5655	cod; D19/5/00
4335	18	-270	M	34	75	0.78	1	31/12/99	15/5/00	5659	D26/5/00
4334	18/22	-120	M	35	77	0.71	31	1/1/00	15/5/00	5660	D26/5/00
4333	18/22	-270	F	35	76	0.712	27	29/12/99	15/5/00	5661	
4325	18/22	-120	F	37	81	0.837	4	21/12/99	15/5/00	5662	
4324	18/22	-120	F	36	78	0.852	23	31/12/99	15/5/00	5663	cos
4323	18/22	-120	M	33	76	0.728	40	8/1/00	16/5/00	5664	D28/03/01
4255	18/22	-120	F	32	71	0.74	17	6/1/00	16/5/00	5665	cos; D25/5/00
4254	18/22	-120	M	35	73	0.712	46	4/1/00	17/5/00	5666	D10/12/00
4253	18/22	-120	M	33	70	0.694	38	27/12/99	17/5/00	5667	
4252	18/22	-120	F	34	74	0.749	32	24/12/99	18/5/00	5669	cos
4245	18/22	-120	M	35	71	0.731	51	4/1/00	18/5/00	5670	cos
4244	18/22	-270	F	33	71	0.702	47	6/1/00	18/5/00	5671	D30/6/00
4243	18/22	-270	M	32	76	0.602	12	6/1/00	19/5/00	5672	D?/11/00
4242	18/22	-120	M	34	75	0.75	9	3/1/00	19/5/00	5673	D28/5/00
4235	18/22	-270	M	35	75	0.767	56	31/12/99	20/5/00	5674	D13/12/00
4234	18/22	-120	F	35	77	0.823	50	6/1/00	20/5/00	5675	
4233	18/22	-270	M	36	81	0.891	42	23/12/99	20/5/00	5676	D11/8/00
4232	18/22	-270	M	33	70	0.632	57	6/1/00	21/5/00	5677	
4225	18	-270	M	34	75	0.718	45	6/1/00	22/5/00	5679	
4224	18	-120	M	34	73	0.75	25	3/1/00	22/5/00	5680	cos; D7/6/00
4223	18	-120	F	34	76	0.76	19	2/1/00	22/5/00	5681	cos; D9/6/00
4222	18	-120	F	33	75	0.74	20	29/12/99	23/5/00	5682	cos; D11/6/00
3555	18	-120	F	34	73	0.785	8	6/1/00	24/5/00	5683	D15/6/00
3554	18/22	-270	F	33	71	0.794	53	3/1/00	24/5/00	5684	cos
3552	18/22	-270	M	33	70	0.723	43	23/12/99	24/5/00	5685	cos; D28/7/00
3544	18/22	-270	M	34	71	0.781	18	17/1/00	25/5/00	5686	
3543	18	-270	F	33	73	0.61	13	2/1/00	25/5/00	5690	cos; D2/6/00
3542	18	-120	M	34	75	0.754	2	6/1/00	26/5/00	5691	D10/12/00
3535	18	-270	M	34	73	0.775	52	8/1/00	27/5/00	5693	cos; D13/12/00
3534	18/22	-120	F	35	74	0.728	58	14/1/00	29/5/00	5696	D12/2/00
3533	18	-120	M	35	74	0.823	6	3/1/00	30/5/00	5697	D14/6/00
3532	18/22	-120	F	34	71	0.68	33	6/1/00	30/5/00	5698	D27/10/00
3525	18	-270	M	34	75	0.739	44	12/1/00	31/5/00	5700	D9/8/00
3524	18	-270	M	32	67	0.692	59	19/1/00	3/6/00	5702	
3523	18	-120	F	35	77	0.735	21	15/1/00	4/6/00	5703	

Abbreviations used

T-C = toe clip – read RF, LF, RR, LR viewed from dorsal surface (Figure I), RF = right front, LF = left front, RR = right rear, LR = left rear.

T = temperature (°C) that individual was incubated.

WP = water potential (kPa) that individual was incubated.

Sex = sex of individual; F = female, M = male.

SVL = the length (mm) from snout to cloacal opening (vent) at hatching.

TL = the length (mm) from snout to tail tip at hatching.

Wt = the mass (g) without umbilical cord etc. at hatching.

DateL = the date the egg was laid.

DateH = the date the juvenile hatched.

FT = frozen tissue number(s) assigned to individual.

cos = cut out of egg as sweating for at least 2 days.

cod = cut out of egg to check on development.

D<date> = individual died on <date>.

Dash (-) = not applicable/not determinable.

Dates are all written day/month/year.

APPENDIX II

Histology

A) Fixation and storage

Eggs

Two eggs were fixed immediately after oviposition to determine the embryonic stage at oviposition. This entailed:

1) Soaking in fixative; 50 g picric acid, 850 ml Diethylene dioxide (1,4-Dioxan $CH_2.CH_2.O.(CH_2)_2.O$), 100 ml formalin, 50 ml Formic acid (two days).
2) Storing in dioxan.

Any eggs that dehydrated or went mouldy were assigned FT numbers and frozen at -80°C (Sanyo™ Vip series -86°C).

Embryos and hatchlings

Fully developed embryos and hatchlings that died naturally were preserved by:

1. Cutting into the abdominal cavity through ventral skin (embryo tails also removed),
2. Fixing specimen in neutral buffered formalin; 100 ml Formaldehyde solution (37-40%); 900 ml distilled water, 4 g $NaH_2PO_4.H_2O$, 6.5 g $NaHPO_4$ (three days),
3. Storing in 70% ethanol.

Eggshell, egg fluid and tails were assigned FT numbers and frozen at -80°C.

B) Embedding

Before embedding was undertaken, the position of gonads was determined with the use of a dissecting microscope (Olympus™ model SD-ILK, SD30). The specimen was then severed about 2 mm below the cloacal opening and about 1 mm above the gonad. The section containing the gonads was labelled and stored in 70% ethanol in soda glass specimen tubes (50 x 18 mm). The head and thorax section (and complete juveniles that died after 4 months of age) were stored in 70% ethanol, whilst the hind legs and tail were discarded. The sectioned piece containing the gonads was then embedded.

Detailed embedding schedule

Soak specimens separately in vials of solution:

1. 70-80% ethanol (one hour),
2. 80-95% ethanol (two hours),
3. 95% ethanol (two hours),
4. absolute ethanol (two hours),
5. ½ absolute ethanol and ½ xylol mixture (two hours),
6. concentrated xylol (two hours),
7. fresh concentrated xylol (two hours),
8. ½ xylol and ½ wax mixture (two hours),
9. pure wax (cooled at room temperature over night).

The following day the specimens in wax (Paraplast tissue embedding medium, 56°C) were reheated in a Contherm oven to melting (56°C). The specimens were then lodged in a wax capsule on a pine block using cooled copper elbows. A paper label was stuck between the wax capsule containing the specimen, and the pine block.

C) Sectioning and staining

Sectioning of block

The embedded block of tissue was sectioned to 7 μm using a metal wedge microtome. The sections were floated in a warm water bath, below the temperature to melt wax, and onto a slide. The slides were dried in a 30°C Contherm oven for at least two days, and then stained with Haematoxylin and Eosin.

Detailed staining schedule for light microscopy

Soak slides in baths of solution:

1. histoclear (five minutes),
2. 100% isopropanol (three minutes),
3. 100% isopropanol (three minutes),

For the rest of the staining process, the slides were put into an automated staining machine (Shandon Elliott), which soaked slides in the following baths of solution:

4. 70% isopropanol (two minutes),
5. 50% isopropanol (two minutes),
6. distilled water (two minutes),
7. Delafield's Haematoxylin (20 minutes),
8. tap water (three minutes),
9. acid/alcohol mix - 1% HCl and 95 % ethanol (10 seconds),
10. salt/water mix - tap water and 20 drops of lithium carbonate (three minutes),
11. aqueous eosin (five minutes),
12. tap water (five minutes),
13. 95 % isopropanol (one minute),
14. 95% isopropanol (two minutes),
15. 100% isopropanol (two minutes),
16. 100% isopropanol (two minutes),
17. histoclear (two minutes),
18. histoclear (seven minutes).

After staining, slides were mounted using duplex mounting media and various sized cover slips.

APPENDIX III

Results from statistical analyses

A) Chapter 2 results

Table IIIa Results from statistical analyses of maternal effects, incubation period and egg mass. A list is present on page 74 describing abbreviations used.

Test	Independent Variable	Dependent Variable	Covariate	df	Mean Square	F-statistic	p-value
Maternal effects (ANOVA)	Maternal SVL	Mean egg mass	-	1	8.696	0.827	0.367
	Maternal SVL	Number of eggs	-	3	62.901	5.981	0.001
	Number of eggs	Mean egg mass	-	3	8.781^{-03}	1.085	0.363
Incubation Period (univariate GLM)	Incubation regime	Days until hatching	Initial egg mass	1	7.897	0.577	0.449
	Incubation temperature	"	"	2	78253.848	5715.287	<0.001
	Water potential	"	"	1	6.808	0.497	0.482
	Incubation temperature × water potential	"	"	2	1.651	0.121	0.887
Change in Egg Mass Over Incubation Period (ANOVA)	Incubation regime	Change in egg mass	Initial egg mass	1	0.706	5.819	0.017
	Incubation temperature	"	"	2	1.037	8.551	<0.001
	Water potential	"	"	1	4.143	34.150	<0.001
	Incubation temperature × water potential	"	"	2	0.343	2.828	0.062

Table IIIb Results from statistical analysis of hatching success. A list is present on page 74 describing abbreviations used.

Test	Independent Variable	Dependent Variable	Covariate	df	Residual Deviance	χ^2	p-value
Hatching Success (logistic regression)	Incubation temperature	Hatch or not	-	171,2	177.1	8.020	0.018
	Water potential	"	-	172,1	184.3	0.828	0.363
	Initial egg mass	"	-	172,1	184.2	0.983	0.322
	Incubation temperature × water potential	"	-	170,3	176.3	0.878	0.349
	Incubation temperature × initial egg mass	"	-	170,3	176.1	1.059	0.303
	Incubation temperature × water potential × initial egg mass	"	-	169,4	175.3	0.935	0.386

B) Chapter 3 results

Table IIIc Results from statistical analysis of hatchling size and condition index. A list is present on page 74 describing abbreviations used.

Test	Independent Variable	Dependent Variable	Covariates	df	Mean Square	F-statistic	p-value
Hatchling Size (multivariate GLM)	Sex	Size measures	Initial egg mass	4	-	0.232	0.920
	Incubation temperature	SVL	"	2	18.086	29.966	<0.001
		VTL	"	2	91.325	21.986	<0.001
		TL	"	2	182.534	31.823	<0.001
		Wt	"	2	1.094^{-02}	5.087	0.008
	Water potential	SVL	"	1	4.627	6.900	0.010
		VTL	"	1	36.899	8.883	0.004
		TL	"	1	67.660	11.796	0.001
		Wt	"	1	1.664^{-02}	7.742	0.006
	Incubation regime	SVL	"	1	38.385	57.233	<0.001
		VTL	"	1	60.711	14.616	<0.001
		TL	"	1	195.645	34.109	<0.001
		Wt	"	1	0.153	71.125	<0.001
	Incubation temperature × water potential	SVL	"	2	0.294	0.439	0.646
		VTL	"	2	6.912	1.664	0.194
		TL	"	2	4.855	0.846	0.432
		Wt	"	2	5.601^{-05}	0.026	0.974
Change in Condition Index Over Four Months (ANOVA)	Incubation temperature	Change in condition	Initial egg mass	2	9.407^{-05}	51.913	<0.001
	Water potential	"	"	1	2.720^{-06}	1.501	0.223
	Incubation regime	"	"	1	1.424^{-06}	0.777	0.380
	Incubation temperature × water potential	"	"	2	4.149^{-07}	0.229	0.796

Table IIId Results from statistical analysis of growth and locomotor performance. A list is present on page 74 describing abbreviations used.

Test	Within-subject Variables	Between-subject Variable	Covariates	df	Mean Square	F-statistic	p-value
Growth to Four Months (repeated measures GLM)	Month measures	Incubation temperature	Hatchling size (SVL, VTL, TL, Wt)	18	-	150.399	<0.001
	SVL			2	286.671	150.399	<0.001
	VTL			2	1087.590	143.612	<0.001
	TL			2	2487.917	180.102	<0.001
	Wt			2	286.671	150.399	<0.001
	Month measures	Water potential	"	9	-	1.786	0.067
		Sex	"	9	-	0.599	0.798
		Initial egg mass	"	9	-	1.784	0.069
		Hatching SVL	"	9	-	0.405	0.933
		Hatching VTL	"	9	-	1.422	0.175
		Hatching TL	"	9	-	1.227	0.246
		Hatching Wt	"	9	-	1.500	0.144
		Incubation temperature × water potential	"	18	-	1.394	0.127
Locomotor Performance (repeated measures GLM)	Month	Incubation temperature	All below	2	-	2.407	0.097
		Water potential	"	1	-	0.176	0.676
		Incubation regime	Sex	1	-	0.065	0.799
			Initial egg mass	1	-	0.001	0.978
			Maternal SVL	1	-	0.230	0.633
			SVL	1	-	0.376	0.542
			TL	1	-	0.001	0.978
			Wt	1	-	2.303	0.133
			LL	1	-	0.348	0.557
			FL	1	-	0.017	0.332
			Condition	1	-	4.020	0.048

Test	Within-subject Variables	Between-subject Variable	Covariates	df	Mean Square	F-statistic	p-value
Locomotor Performance (repeated measures GLM)	Month	Incubation temperature × water potential	All below	2	-	0.037	0.903
	Sprint temperature	Incubation temperature	"	4	-	2.482	0.046
		Water potential	"	2	-	0.454	0.637
		Incubation regime	Sex	2	-	1.097	0.339
			Initial egg mass	2	-	2.151	0.123
			Maternal SVL	2	-	0.178	0.838
			SVL	2	-	0.256	0.775
			TL	2	-	0.073	0.930
			Wt	2	-	0.776	0.464
			LL	2	-	0.380	0.685
			FL	2	-	0.279	0.757
			Condition	2	-	5.114	0.008
	Month × sprint temperature	Incubation temperature	All below	4	-	2.149	0.077
		Water potential	"	2	-	0.725	0.488
		Incubation regime	Sex	2	-	0.416	0.661
			Initial egg mass	2	-	6.968	0.002
			Maternal SVL	2	-	0.074	0.929
			SVL	2	-	1.161	0.319
			TL	2	-	0.186	0.831
			Wt	2	-	1.378	0.258
			LL	2	-	0.866	0.425
			FL	2	-	0.314	0.732
			Condition	2	-	4.723	0.012
		Incubation temperature × water potential	All above	4	-	1.164	0.329

Table IIIe Results from statistical analysis of survival. A list is present below this table describing abbreviations used.

Test	Independent Variable	Dependent Variable	Covariate	df	Residual Deviance	χ^2	p-value
Survival to Four Months (logistic regression)	Incubation temperature	Survive or not	-	120,2	59.4	43.023	<0.001
	Water potential	"	-	121,1	102.4	0.001	0.970
	Sex	"	-	121,1	101.9	0.475	0.491
	Initial egg mass	"	-	121,1	101.8	0.622	0.431
	Incubation temperature × water potential	"	-	119,3	59.3	0.010	0.748
	Incubation temperature × sex	"	-	119,3	59.3	0.125	0.724
	Incubation temperature × initial egg mass	"	-	119,3	56.7	2.666	0.103

Abbreviations used

$\chi 2$ = chi square statistic.

df = degrees of freedom.

FL = foot length.

GLM = general linear model.

LL = leg length.

SVL = snout-vent length.

TL = total length.

VTL = vent-tail length.

Wt = mass.