**Temperature-dependent Sex Determination
and Artificial Incubation of Tuatara,
*Sphenodon punctatus***

Nicola J. Nelson

A thesis submitted for the degree of
Doctor of Philosophy in Zoology
Victoria University of Wellington
Te Whare Wananga o te Upoko o te Ika a Maui
2001

Abstract

Juveniles resulting from artificially induced and incubated eggs are often used to found or augment populations of rare reptiles, but both procedures may compromise the health of hatchlings or their fitness in natural environments. I aimed to test whether these procedures affected size or performance of juvenile tuatara, *Sphenodon punctatus*, New Zealand reptiles with temperature-dependent sex determination (TSD). Size and performance are phenotypic traits likely to influence fitness and eventual lifetime reproductive success, and are thus important measures of the suitability of artificial induction and incubation techniques for conservation management.

I incubated 320 tuatara eggs artificially at 18, 21 and 22°C; 52% of these were obtained by induction, the remainder were collected from natural nests. An additional 25 natural nests were left intact for investigation of TSD and effects of incubation temperature in nature. Juveniles from all incubation regimes were kept for ten months post-hatching in similar rearing conditions and sexed by laparoscopy.

Induced eggs were significantly smaller than naturally laid eggs, and resulted in significantly smaller hatchlings, even when variation among clutches was accounted for. Incubation temperature did not greatly influence size at hatching, but was an important determinant of size by ten months of age; initial egg mass was the most important factor affecting size of hatchlings.

Data indicate that TSD occurs in nature. The sex of hatchlings from 21 nests was investigated: 10 nests produced 100% male hatchlings, 4 nests produced 100% female

hatchlings, and only 7 nests produced mixed sex ratios which ranged from 11% to 88% males. Sex of juveniles was related to temperature with a larger proportion of males produced in warmer nests. The overall percentage of male hatchlings in natural nests was 64%. Hatching success was 65% from natural nests during the 1998/99 season. Incubation temperatures throughout the year ranged from 2.9 to 34.4°C. Global warming is likely to skew the hatchling sex ratio towards males if female tuatara are unable to select nest sites according to environmental cues. Evidence from size patterns of tuatara incubated in natural nests supports differential fitness models for the adaptive significance of TSD.

The evaluation of artificial incubation as a conservation management tool demonstrated that it is a procedure that benefits conservation as it can be used reliably to produce founders; hatching success was 94% during this study. The sex ratio of artificially incubated juveniles can be easily manipulated; the pivotal temperature lies between 21 and 22°C. Constant artificial incubation conditions resulted in larger juveniles by ten months of age than those from natural incubation. Naturally incubated juvenile tuatara, however, were faster for their size, their reaction norm to predator stimuli was to run, and they were possibly more aggressive, suggesting naturally incubated juveniles could survive better in nature. No firm conclusions can be reached on the quality of artificially incubated juvenile tuatara because further research will be required to establish the relevance of performance test results in nature and consequences of incubation regimes in the longer term with respect to relative fitness of individuals.

Acknowledgements

Over the past three years I've had tremendous help and support from a huge number of enthusiastic people. I've enjoyed their company and hope I've done justice to their efforts by finally completing the project. Most importantly, I couldn't have done it without the fantastic support and patience of my family, in particular, Craig and Josh. Special thanks to Shirley Pledger for her time and invaluable statistical advice, Mike Thompson for endless advice and manuscript tampering, Alison Cree for getting me started on sexing tuatara, Bryan Gartrell for veterinary advice, training and equipment, Geoff Birchard for discussions and interesting sideline experiments, Sue Keall for endless help in all facets of the project, and Charlie Daugherty for being a great supervisor and 'precising' my thesis. The collection of information in the field and laboratory, and reviewing of manuscripts was achieved with the generous help of the following people: Dick Brandon, Scott Carver, Margaret Crimp, Dale DeNardo, Mike Dickison, Ana Djorovic, Sharon Downs, Jim Elkington, Peter Gaze, Kelly Hare, Tiwini Hippolite, Edith Hodgen, Tonia Hsieh, Bronwyn Kivell, Christa Krey, Chris Longson, Tim Markwell, Katrina Merrifield, John Miller, Nicki Mitchell, Richard Moore, Christa Mulder, Anaru Paul, Polly Phillpot, Brett Robertson, Bryan Rowband, Bryan Simpkins, Andrew Styche, Dennis Thompson, Chris Thorn, Karen Waller, Steve Ward, Peter Watson, Corrinne Watts, and Tony Whitaker. Thanks to everyone at Nga Manu Nature Reserve, and to Arthur Georges who generously allowed me to use his models and computer programmes. I'm grateful for financial support provided by the Marsden Fund (Grant Number: 98-MAU-005) and Victoria University of Wellington. Dr Stephen Sarre and Prof David Lambert (Massey University) were collaborators in the Marsden proposal, and provided invaluable assistance throughout the duration of the project. This study was carried out under Department of Conservation permit numbers TAK 98/8 and LIZ 98/6, and Victoria University of Wellington Animal Ethics Committee approval numbers CD00R1 and CD98R1. Ngati Koata, as tangata whenua of Takapourewa (Stephens Island), approved the research programme.

Table of Contents

Abstract	i
Acknowledgments	iii
Table of Contents	iv

CHAPTER 1. GENERAL INTRODUCTION 1

CHAPTER 2. INDUCTION OF OVIPOSITION PRODUCES SMALLER EGGS IN TUATARA (*SPHENODON PUNCTATUS*) 5

2.1. ABSTRACT.....	5
2.2. INTRODUCTION.....	6
2.3. METHODS.....	7
2.3.1. Statistical Analyses	10
2.4. RESULTS	11
2.5. DISCUSSION	14
2.5.1. Clutch Size	14
2.5.2. Initial Egg Mass	15
2.5.3. Available Calcium and Body Size	17
2.5.4. When To Induce.....	18
2.6. REFERENCES	19

CHAPTER 3. MALE BIASED SEX RATIO AMONG HATCHLINGS FROM NATURAL TUATARA NESTS 22

3.1. ABSTRACT.....	22
3.2. INTRODUCTION.....	23
3.3. METHODS.....	25
3.3.1. Statistical Analyses	29
3.4. RESULTS	31
3.4.1. Natural Nest Characteristics, Incubation Duration, and Hatching Success	31
3.4.2. Temperature	32
3.4.3. Sex Ratio	32
3.4.4. Temperature and Sex Ratio	34
3.5. DISCUSSION	35
3.6. REFERENCES	40

CHAPTER 4. EGG MASS GOVERNS HATCHLING SIZE, BUT INCUBATION REGIME INFLUENCES POST-HATCHING GROWTH OF TUATARA 43

4.1. ABSTRACT.....	43
4.2. INTRODUCTION.....	44
4.3. METHODS.....	47

4.3.1. Statistical Analyses	50
4.4. RESULTS	52
4.4.1. Duration of Incubation	53
4.4.2. Size at Hatching and Ten Months	54
4.4.3. Effects of Artificial Incubation Temperature on Sex	54
4.4.4. Effects of Artificial Incubation on Tuatara Size	54
4.4.5. Effects of Natural Incubation on Tuatara Size	65
4.4.6. Size of Tuatara: Natural Versus Artificial Incubation	69
4.5. DISCUSSION	69
4.5.1. Influences on Size of Tuatara	69
4.5.2. Consequences of Size Differences for Tuatara	74
4.5.3. Artificial Versus Natural Incubation	74
4.5.4. Sexual Dimorphism	76
4.5.5. Summary	76
4.6. REFERENCES	78

CHAPTER 5. PERFORMANCE AND POTENTIALLY FITNESS OF TUATARA DEPEND ON INCUBATION REGIME AND LENGTH OF JUVENILES85

5.1. ABSTRACT	85
5.2. INTRODUCTION	86
5.3. METHODS	89
5.3.1. Source Animals, Test Times and Conditions	89
5.3.2. Performance Measures	90
5.3.3. Statistical Analyses	91
5.4. RESULTS	94
5.4.1. Sprint Speed	94
5.4.2. Predator Avoidance	99
5.4.3. Willingness to Feed	103
5.5. DISCUSSION	107
5.5.1. Performance of Juvenile Tuatara	108
5.5.2. Suitability of Performance Tests	109
5.5.3. Interpretation of Performance Results for Fitness	110
5.5.4. Competence of Artificially Incubated Tuatara	112
5.6. REFERENCES	113

CHAPTER 6. QUALITY CONTROL: ARE ARTIFICIALLY INCUBATED TUATARA UP TO THE STANDARD SET BY NATURAL INCUBATION?117

6.1. INTRODUCTION	117
6.2. REVIEW OF RESULTS: CHAPTERS 2-5	117
6.2.1. Induction of Oviposition	117
6.2.2. Incubation Regime	118
6.3. DISCUSSION	119
6.4. RECOMMENDATIONS FOR FUTURE RESEARCH	120
6.5. REFERENCES	123

Chapter 1. General Introduction

This thesis investigates the significance of temperature-dependent sex determination (TSD) and artificial incubation for tuatara, *Sphenodon punctatus*. It is written as a series of independent manuscripts for publication. Some repetition is unavoidable for this reason. Co-authors are acknowledged for each chapter/manuscript. I take primary responsibility for all methodology, data collection, analyses, and writing of this thesis.

This project was part of a collaboration to investigate the existence and phenotypic consequences of TSD and genetic sex determination (GSD) in tuatara. This thesis presents only results of incubation experiments to investigate TSD and phenotypic consequences of artificial and natural incubation regimes for tuatara. At the time of submission of this thesis, the existence of GSD in tuatara was still under investigation. Analyses presented here do not include this aspect of the original design.

Tuatara eggs were incubated in two main treatment groups: at constant temperatures in the laboratory, or at variable temperatures in natural nests. Eggs were collected for artificial incubation by hormonal induction of oviposition or recovered from natural nests. All hatchlings were reared in similar artificial conditions, and performance of hatchlings was periodically measured (Figure 1). This resulted in an overall experimental design comprising three treatment groups:

- (i) tuatara hatchlings from eggs that were hormonally induced and then artificially incubated;

- (ii) hatchlings from eggs that were laid naturally in nests then removed for artificial incubation;
- (iii) hatchlings from eggs that were laid and incubated naturally in nests, and removed just at the time of hatching.

Figure 1. The experimental design comprising three treatment groups: (i) tuatara hatchlings from eggs that were hormonally induced then artificially incubated; (ii) hatchlings from eggs that were laid naturally in nests then removed for artificial incubation; (iii) hatchlings from eggs that were laid and incubated naturally in nests.

In Chapter Two I describe the methodology used for artificial incubation regimes and the effect of induction as a collection method on size of tuatara eggs and hatchlings. I describe the methodology used for the natural incubation regime throughout the thesis in Chapter Three and present evidence for the existence of TSD in tuatara in nature. In Chapter Four I compare and analyse the effects of incubation regimes presented in Chapters Two and Three on size of hatchlings. In Chapter Five I investigate performance of those juvenile tuatara with respect to size and incubation regime. The significance of the results of Chapters Two to Five is discussed in Chapter Six, with emphasis on artificial versus natural techniques for incubation of tuatara, and areas for future research.

The artificial incubation treatments (constant 18, 21 and 22°C) were selected for three reasons:

- (i) All three temperatures were expected to produce hatchlings with high success (Thompson 1990, Cree et al. 1995).
- (ii) Information was available on expected sex ratios: the 18°C treatment was expected to produce 100% females; the 21°C treatment was expected to produce close to a 1:1 sex ratio; the 22°C treatment was expected to produce both sexes, but with a higher proportion of males (Cree et al. 1995). This potentially allowed for comparison of effects of temperature on performance while controlling for effects of sex.
- (iii) The selected temperatures encompass the range used for incubation of tuatara eggs to produce founders for new populations and to augment rare populations.

I chose not to extend the range of temperatures lower than 18°C, or higher than 22°C as previous research suggested hatching success would be compromised (Thompson 1990). I chose not to use variable artificial incubation temperature treatments due to equipment limitations and the need to simplify treatments as much as possible to allow meaningful analyses to be conducted.

References:

- Cree, A., Thompson, M. B. and C. H. Daugherty. 1995. Tuatara sex determination. *Nature* 375:543.
- Thompson, M. B. 1990. Incubation of eggs of tuatara, *Sphenodon punctatus*. *Journal of Zoology, London* 222:303-318.

Chapter 2. Induction of oviposition produces smaller eggs in tuatara (*Sphenodon punctatus*)

Nicola J. Nelson¹, Michael B. Thompson², Shirley Pledger³, Susan N. Keall¹
and Charles H. Daugherty¹

¹ School of Biological Sciences, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand; ² School of Biological Sciences and Wildlife Research Institute, Heydon-Laurence Building (A08), University of Sydney, NSW 2006, Australia; ³ School of Mathematics and Computing Science, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand.

2.1. Abstract

Induction of oviposition by injection of hormones has been used successfully to collect eggs from at least three orders of reptiles. Eggs of tuatara (*Sphenodon* spp.) have been collected by induction since 1985. Collection of eggs from tuatara nests takes considerably longer and relies on knowledge of nesting areas and timing. We investigate whether inducing egg laying affects clutch size, egg mass, hatching success or hatchling size, and describe considerations necessary when deciding whether to induce egg laying or collect eggs from nests. Tuatara (*S. punctatus*) eggs were collected for incubation from Stephens Island in 1998: 52% by induction and 48% from nests. Induced clutches were significantly larger than naturally laid clutches, probably because not all eggs from naturally laid clutches were located. Induced eggs were significantly smaller than naturally laid eggs, even when variation among clutches was accounted for. Incubation temperature was the only significant factor influencing the probability of hatching. Induced eggs resulted in significantly smaller hatchlings following incubation in captivity, and the difference was still significant when hatchlings were 10 months old. Induction is an extremely useful technique for obtaining eggs of tuatara and other reptiles, but it must be used with caution as it reduces hatchling size in tuatara.

Keywords: *Sphenodon punctatus*, tuatara, oviposition, induction, egg size, incubation.

2.2. Introduction

Induction of oviposition by injection of hormones has been used successfully to collect eggs from at least 3 orders of reptiles (Ewert and Legler 1978, Guillette and Jones 1982, Packard et al. 1985, Thompson et al. 1991). Induction is viewed as a useful alternative to sacrificing gravid females or taking eggs from nests because it is non-destructive, apparently harmless, inexpensive, and a relatively simple procedure (Cree et al. 1991, Thompson et al. 1991). The risks of induction include potential effects of hormones on the physiology of the mothers or their eggs, or poorly calcified eggs if oviposition is premature.

Tuatara (*Sphenodon* spp.) are biologically significant as the sole living representatives of the reptilian order Sphenodontia (*sensu* Benton 1990, Cree and Butler 1993). Now restricted to off-shore islands of New Zealand, they are also of high conservation importance (Cree and Butler 1993); collection of eggs by sacrificing females is not a viable option for these threatened species. Eggs have been collected from tuatara nests but this is more difficult as it is time consuming and relies on knowledge of nesting areas and timing of oviposition. Eggs have been collected using induction by oxytocin since 1985, contributing to studies on laboratory incubation, comparative physiology and sex determination, while producing founders for new captive and wild populations, and augmenting existing rare populations (Cree et al. 1991, Nelson 1998, Thompson 1990, Thompson et al. 1991). Although induction of oviposition in reptiles has been used successfully and as a research tool for two decades, little information is available

on the relative health, survival and reproductive ability of hatchlings resulting from induced eggs compared to those collected by other methods. Induction of tuatara eggs before eggshells have properly formed, results in high mortality (Thompson 1990, Cree et al. 1996). Our research on tuatara has recently required the collection of large numbers of eggs for artificial incubation. We took the opportunity to address the question: does induction of oviposition affect the eggs, embryos or hatchlings? In particular, the aims of this study were firstly to investigate whether induction affects clutch size, egg mass, hatching success or hatchling size, compared to eggs from natural nests, and secondly to describe considerations necessary when deciding whether to induce oviposition.

2.3. Methods

Eggs were collected for incubation from Stephens Island, a 150 ha Nature Reserve in the Marlborough Sounds, New Zealand, which supports the largest population of tuatara (*S. punctatus*; Newman 1982). Between 1-21 November 1998, 320 eggs were collected using two methods: induction of oviposition by injection of synthetic oxytocin (Oxytocin-s, conc. 10 IU/ml, Intervet International BV, Boxmeer, Holland; $n = 166$) and recovery of eggs laid naturally in nests ($n = 154$). November is within the natural egg laying season for tuatara on Stephens Island (Thompson 1990, Thompson et al. 1991).

A total of 26 females identified as gravid by palpation were collected on either 17 or 18 November and taken to a field laboratory for induction. These induction dates were considered to be around the middle of the egg laying season, and hence a representative sample of that season's eggs. Permit and time limitations prevented sampling

throughout the season by induction. Palpation for tuatara eggs was used as a conservative test for assessing gravidity. Females were selected for palpation based on location on or near a rookery. We did not select females for palpation based on size. Discrimination of gravid and non-gravid females is not possible by palpation alone (pers. comm., Alison Cree). Females received an injection of oxytocin (10 IU/kg body mass, shallow intra-peritoneal with a 23G needle) on the evening they were collected, following the protocol of Thompson et al. (1991). Most females were held for up to 24 hours to allow sufficient time to obtain entire clutches. Induction was initially unsuccessful in two females, which were then held for 48 hours. One of these females did not lay eggs. Eggs from four clutches were small (<3 g) and misshapen. These were buried in nesting rookeries on the island and not included in further analyses.

Female nesting activity was monitored twice daily over the three week collection period in known rookeries (Cree and Thompson 1988), and potential nests were marked. Most eggs were collected from natural nests within 24 hours of oviposition but, due to the difficulty of inspecting nest chambers without destroying them, oviposition dates for ten naturally laid clutches were retrospectively calculated at 4-9 days prior to collection. Any eggs that were very small (<3 g), misshapen (for example, collapsed) or infected with fungi were presumed unlikely to hatch and were reburied on the island.

All eggs were assigned a unique number written on the top surface of the egg with a soft (4B) graphite pencil, and the orientation of the egg was maintained throughout the study (Thompson 1990). Eggs were weighed to 1 mg on a Sartorius (GMBH Type 1475, Gottengen, Germany) top pan balance on the day of collection and placed half buried in moist vermiculite (approx. -170 kPa) at room temperature on the island (approx. 18-

22°C). On 21 November 1998, eggs collected by both methods were flown by helicopter to Victoria University of Wellington for incubation.

Each incubation box comprised a sealed 2 L plastic container half-filled with vermiculite and water (-170 kPa), and contained four induced and four naturally laid eggs. Clutches were divided into three groups and each group was randomly assigned to one of three constant incubation temperatures (18, 21 and 22°C). Eggs were then randomly assigned to an incubation box. Eggs were weighed weekly, when distilled water was added to incubation boxes to make up for small losses from the container and uptake by the eggs, thus maintaining a water potential of approximately -170 kPa. Incubators were calibrated prior to use, and incubation temperatures were checked daily. Temperatures were recorded by dataloggers (waterproof Stowaway[®] Tidbit[®] temperature recorders). Incubation boxes were moved within each incubation chamber daily. Hatchlings were weighed, measured (snout-vent length (SVL), total length, head width and jaw length), and individually marked with a toe-clip on the day they hatched, and weighed and measured monthly until 10 months of age. The only juvenile measurement used in analyses carried out in this chapter was SVL.

Hatchlings were reared in similar conditions, with 16 individuals per 600 x 700 mm enclosure. Enclosures were furnished with a leaf litter base, tree bark for shelter, and two water dishes. Juveniles were fed three times per week with similar amounts of crickets, house flies or blow flies per enclosure regardless of juvenile size or hatching date. Enclosures were exposed to daily temperature variations of between approximately 18-22°C and a 12:12 light/dark cycle beginning at 0600 h throughout the

year (Duro-test[®] True-lite[®] power twist fluorescent tubes, placed 750 mm above the enclosure).

2.3.1. Statistical Analyses

S-plus and Systat 10.0 statistical programmes were used for data analyses. The effect of induction on clutch size was analysed using a Mann Whitney-*U* rank sum test. Effects of induction and clutch identity on initial egg mass were analysed using a nested analysis of variance (ANOVA). Clutch identity was treated as a random effect nested within collection method (that is, induced versus naturally laid).

The effects of induction and clutch identity on hatchling SVL and SVL at ten months of age were tested using analyses of covariance (ANCOVA), with initial egg mass as a covariate. Other covariates investigated included last egg mass prior to hatching, gain in egg weight during incubation, and incubation time. Fixed factors included incubation temperature and collection method. Dummy variables for class variables were constructed automatically by the statistical packages. Clutch identity was treated as a random effect nested within collection method. For both SVL response variables, we used F-tests ($p < 0.05$) in a stepwise procedure to compare these general linear models. As clutch was nested in collection method, any model including a clutch effect (on $c-1$ df where there are c clutches) may have the effect separated into a collection method effect (1 df) and a clutch-within-method effect ($c-2$ df). This provided a 1 df test for a collection method effect over and above any clutch-within-method effect and other effects in the model (Searle 1987).

We acknowledge a problem of pseudo-replication with respect to incubation temperature as only one incubator was used per temperature treatment. This is, however a standard technique for artificial incubation of reptilian eggs. Incubation box effects were not significant and were left out of all future analyses. Enclosure effects could not be allowed for due to the limitations imposed on the experimental design by highly variable hatching dates among temperature treatments.

A logistic regression modelling the probability of hatching, using a stepwise process for model selection ($p < 0.05$), was carried out to analyse hatching success with possible explanatory variables of collection method, initial egg mass, clutch and incubation temperature. Models were compared using a chi-square test, where the test statistic was equal to the change in residual deviance and degrees of freedom, respectively, between the two models.

2.4. Results

Eggs were collected from 50 females. Mean size of induced clutches (9.1 eggs; $n = 21$) was significantly larger than naturally laid clutches (6.3 eggs; $n = 29$; $T = 689.0$, $p = 0.001$; Table 1). The smallest induced clutch contained five eggs compared to only one egg in the smallest naturally laid clutch (Table 1).

Mean individual egg mass at oviposition for induced eggs (4.498 g, $n = 191$) was significantly smaller than for naturally laid eggs (5.375 g, $n = 183$; Table 2; $F_{(1, 48)} = 14.94$, $p \leq 0.01$) after allowing for possible clutch effects. Clutch effects were significant ($F_{(48, 324)} = 8.58$, $p \leq 0.01$), accounting for 50% of the remaining variance.

Initial egg mass was not significantly correlated with clutch size ($F_{(1)} = 3.44$, $p = 0.0698$).

Hatching success was high: 94% of induced eggs and 93% of naturally laid eggs hatched. Incubation temperature was the only factor significantly influencing the probability of hatching ($\chi^2 = 15.59$, $df = 2$, $p = 0.0004$); hatching success was lower at 18°C.

Table 1. Size of induced and naturally laid tuatara egg clutches.

Collection Method	No. of Clutches	Mean Clutch Size	SE	Range (No. eggs)
Induction	21	9.1	0.4	5 - 13
Natural	29	6.3	0.7	1 - 14
Total	50	7.6		

Table 2. Individual egg mass at oviposition (including those eggs not incubated).

Collection Method	No. of Eggs	Mean Initial Egg Mass (g)	SE	Range (g)
Induction	191	4.498	0.06	1.388 - 6.223
Natural	183	5.375	0.07	1.598 - 8.023

Eggs collected by induction produced significantly smaller hatchlings than eggs from natural nests: 52.2 mm SVL versus 54.1 mm ($T = 23549$, $p \leq 0.001$; Table 3). The effect of collection method was mediated through initial egg mass, in that models including egg mass (the most important predictor of hatchling size) no longer needed to include the (correlated) collection method ($p = 0.469$; Figure 1). Other important predictors of hatchling SVL were clutch identity, incubation temperature and incubation time (stepwise regression $p < 0.05$). These factors are addressed fully in Chapter 4. For juveniles at 10 months of age, SVL had the same four predictors, but with less predictive power (higher p values), and in a different order of importance: incubation temperature, initial egg mass, clutch identity and then incubation time. Again, with initial egg mass and clutch identity in the model, collection method added no useful predictive information ($p = 0.952$).

Table 3. Size of tuatara at hatching and at 10 months.

Age	Collection Method	No. of Hatchlings	Mean SVL (mm)	SE	Range (mm)
Hatchlings	Induction	155	52.2	0.2	46 - 57
	Natural	130	54.1	0.2	45 - 59
10 month old juveniles	Induction	152	82.4	0.4	64 - 94
	Natural	130	84.8	0.4	72 - 95

Figure 1. The relationship of tuatara egg mass and hatchling size. Naturally laid eggs (open circles) were significantly larger than induced eggs (crosses), and larger initial egg mass resulted in larger hatchling size.

2.5. Discussion

Induction of tuatara eggs resulted in significantly larger clutches, but smaller initial egg mass and hatchling size. Hatching success and subsequent survival were not significantly affected by induction. In comparison, the method of procurement of turtle eggs (*Chelydra serpentina*) had no effect on hatching success or hatchling body size. However, female turtles were induced to lay directly into water (Wilgenbusch and Gantz 2000).

2.5.1. Clutch Size

The size range of induced clutches in this study (5-13 eggs) is similar to that found by previous work on *S. punctatus* from Stephens Island of 1-18 eggs using x-rays

(Newman et al. 1994). Hence, the difference in size of induced and natural clutches described here may be an artifact of collection method. Occasionally, eggs are found outside natural nests in the field, and female tuatara often dig up nests of other females during the nesting season (Cree and Thompson 1988), suggesting that despite intensive searching, we obtained only partial clutches from natural nests. We do not know whether females that deposit only partial clutches in natural nests go on to construct other nests. Alternatively, smaller clutch size in naturally laid nests may be due to a difference in SVL of female tuatara producing these eggs, as SVL and clutch size are correlated in tuatara (e.g. Newman et al. 1994). Support for this hypothesis comes from the fact that clutches in nests that were left to incubate naturally (Chapter 3) have comparable numbers of eggs to those of induced clutches suggesting something other than induction was the cause of the difference in clutch size. The relationship between SVL and clutch size was not investigated in this thesis.

2.5.2. Initial Egg Mass

Initial egg mass of induced eggs increases during the nesting season (Thompson 1990; mean = 3.16g in October, 5.06g in December). As both of our treatment groups (natural and induced clutches) came from the same population of female tuatara at the same time, we expected no difference in mean egg size. Possible reasons for a difference in initial egg mass of the two groups include trade-offs between clutch size and egg mass, water content or calcification of eggs. Egg size decreases with increasing clutch size in many species and has been linked to trade-offs associated with constraints on resources and reproductive strategy (e.g., Wallis et al. 1999). The absence of a relationship between clutch size and mean egg mass in this population of tuatara suggests that the smaller eggs in the induced clutches are not a result of larger clutch size. Also, analyses

using only naturally laid clutches of similar size to induced clutches (i.e., five or more eggs) resulted in similar findings (i.e., induced eggs are smaller).

Hatchling size is weakly influenced by the depth that eggs are buried and hence the amount of water absorbed during development (Thompson 1990). However, hatching size is not significantly influenced by incubation at different water potentials (Thompson 1990). Eggs from natural nests were at variable water potentials for up to 9 days prior to collection, while induced eggs were placed immediately into moist vermiculite (-170 kPa). At the room temperatures of the field station and natural water potentials, there is likely to have been little effect of this short period of natural incubation on final egg mass (Thompson 1990, Thompson et al. 1996), as after collection, all eggs had equal access to water and ample time to recoup any water deficit throughout the 6 to 11 month incubation period.

Calcification of tuatara eggs is a slow process spread throughout eight months of gravidity (Cree et al. 1996). Eggs induced in the month prior to nesting may be incompletely calcified (Packard et al. 1988, Thompson 1990), suggesting that calcium may be deposited on the eggshell until laying (Cree et al. 1996). Females found in open areas, on or near rookeries, were selected for induction if abdominal palpation suggested gravidity. As female tuatara only lay eggs about every 4 years (Cree et al. 1991) and it was the nesting season for tuatara on Stephens Island, we assumed that gravid females were almost ready to oviposit. Tuatara can take as long as two weeks to build a nest, so if calcification and 'plumping' is still occurring in the oviduct throughout this period, then eggs of induced females may be less calcified and/or contain less water than

naturally laid eggs and hence weigh less. There was no obvious sign, however, of incomplete calcification in our induced eggs.

2.5.3. Available Calcium and Body Size

Calcium stores in the eggshell have been implicated in ossification of advanced embryos of many reptiles (Jenkins 1975, Simkiss 1967, Packard and Packard 1988, Thompson et al. 2000). Although calcium availability has not to our knowledge been further linked to hatchling size in reptiles, the final length of rat bones depends on ossification (Alvarez et al. 2000), providing a possible mechanism by which calcium deposition late in gravidity might influence hatchling size.

Body size in many organisms is positively correlated with traits that are presumably related to fitness (Congdon et al. 1999). Larger size at hatching affects sprint speed (Sinervo and Adolph 1989, Tsuji et al. 1989), feeding success (Avery et al. 1982), predator avoidance (Christian and Tracy 1981, Webb 1986) and social dominance (Garland et al. 1990) in some hatchling reptiles. Since size of juvenile tuatara at 10 months is still influenced partially by initial egg mass, induction (mediated through initial egg mass) may differentially affect performance of juveniles. In a study of four-year-old tuatara, Goetz and Thomas (1994) found that weight fluctuations were influenced by an animal's social status. Contrary to expectations, the smaller study animal was more socially dominant. With a sample size of two, however, this result is tentative.

2.5.4. *When To Induce*

As induction results in smaller hatchlings and size may be important in performance, many issues must be considered prior to selection of a method of egg collection. Induction is quick and inexpensive, and does not require knowledge of nesting areas. Few field workers are needed to acquire substantial numbers of eggs, although the ability to palpate females for eggs increases the efficiency of the method. Unlike squamates, embryonic development in turtles (Ewert 1979), and apparently tuatara (Moffat 1985), is arrested at about the time of gastrulation and does not resume until after oviposition. Thus, induction of oviposition in turtles and tuatara is a very useful experimental tool because the embryonic stage of oviposition is known precisely. Induction does, however, require knowledge of the nesting season, and premature induction of oviposition results in poorly calcified eggs. Collection of eggs from natural nests requires more time and personnel, and hence is more expensive than induction. Many islands where tuatara are found have limitations on the number of field workers because of the fragile habitat. Knowledge of nesting areas and season are required, and nesting season varies with locality (Tyrrell et al. 2000). However, collection of naturally laid eggs ensures that proper calcification has occurred and that eggs have reached their potential size.

Choice of a method for collecting tuatara eggs depends primarily on knowledge of the ecology of the population being sampled, the reason for collection (e.g. there may be constraints of experimental design), budget, and island characteristics. If induction is the method of choice, it must be used with caution in tuatara, and perhaps other reptiles. Induction reduces hatchling size in tuatara, with unknown consequences for long-term fitness.

2.6. References

- Alvarez, J., Balbin, M., Santos, F., Fernandez, M., Ferrando, S. and J. M. Lopez. 2000. Different bone growth rates are associated with changes in the expression pattern of types II and X collagens and collagenase 3 in proximal growth plates of the rat tibia. *Journal of Bone Mineral Research* 15:82-94.
- Avery, R. A., Bedford, J. D. and C. P. Newcombe. 1982. The role of thermoregulation in lizard biology: predatory efficiency in a temperate diurnal basker. *Behavioural Ecology and Sociobiology* 11:261-267.
- Benton, M. J. 1990. Vertebrate Palaeontology: Biology and Evolution. HarperCollins Academic, London.
- Christian, K. A. and C. R. Tracy. 1981. The effect of the thermal environment on the ability of hatchling Galapagos land iguanas to avoid predators during dispersal. *Oecologia* 49:218-223.
- Congdon, J. D., Nagle, R. D., Dunham, A. E., Beck, C. W., Kinney, O. M. and S. R. Yeomans. 1999. The relationship of body size to survivorship of hatchling snapping turtles (*Chelydra serpentina*): an evaluation of the “bigger is better” hypothesis. *Oecologia* 121:224-235.
- Cree, A. and D. Butler. 1993. Tuatara Recovery Plan (*Sphenodon* spp.). Threatened Species Recovery Plan Series No. 9. Department of Conservation, Wellington, New Zealand.
- Cree, A. and M. B. Thompson. 1988. Unravelling the mysteries of tuatara reproduction. *Forest and Bird* 250:14-16.
- Cree, A., Daugherty, C. H., Schafer, S. F. and D. Brown. 1991. Nesting and clutch size of tuatara (*Sphenodon guntheri*) on North Brother Island, Cook Strait. *Tuatara* 31:9-16.
- Cree, A., Guillette, L. J. Jr. and K. Reader. 1996. Eggshell formation during prolonged gravidity of the tuatara *Sphenodon punctatus*. *Journal of Morphology* 230:129-144.
- Ewert, M. A. 1979. The embryo and its egg: development and natural history. Pp. 333-413. In Harless, M. and H. Morlock (Eds.). *Turtles: perspectives and research*. John Wiley & Sons, New York.
- Ewert, M. A. and J. M. Legler. 1978. Hormonal induction of oviposition in turtles. *Herpetologica* 34:314-318.
- Garland, T., Hankins, J. E. and R. B. Huey. 1990. Locomotor capacity and social dominance in male lizards. *Functional Ecology* 4:243-250.

- Goetz, B. G. R. and B. W. Thomas. 1994. Use of annual growth and activity patterns to assess management procedures for captive tuatara (*Sphenodon punctatus*). *New Zealand Journal of Zoology* 21:473-485.
- Guillette, L. J. Jr. and R. E. Jones. 1982. Further observations on arginine vasotocin - induced oviposition and parturition in lizards. *Journal of Herpetology* 16:140-144.
- Jenkins, N. K. 1975. Chemical composition of the eggs of the crocodile (*Crocodylus novaeguineae*). *Comparative Biochemistry and Physiology* 51A:891-895.
- Moffat, L. A. 1985. Embryonic development and aspects of reproductive biology of the tuatara, *Sphenodon punctatus*. Pp. 493-521. In Gans, C., Billet, F. and P. Maderson (Eds.). *Biology of the Reptilia* 14. John Wiley & Sons, New York.
- Nelson, N. J. 1998. Conservation of Brothers Island Tuatara, *Sphenodon guntheri*. Unpublished MConSc Thesis, Victoria University of Wellington, New Zealand.
- Newman, D. G. 1982. Tuatara, *Sphenodon punctatus*, and burrows, Stephens Island. Pp. 213-221. In Newman, D. G. (Ed.). *New Zealand Herpetology*. New Zealand Wildlife Service Occasional Publication No. 2.
- Newman, D. G., Watson, P. R. and I. McFadden. 1994. Egg production by tuatara on Lady Alice and Stephens Islands. *New Zealand Journal of Zoology* 21:387-398.
- Packard, G. C., Packard, M. J. and W. H. N. Gutzke. 1985. Influence of hydration of the environment on eggs and embryos of the terrestrial turtle *Terrapene ornata*. *Physiological Zoology* 58:564-575.
- Packard, M. J. and G. C. Packard. 1988. Sources of calcium and phosphorus during embryogenesis in bull snakes (*Pituophis melanoleucus*). *Journal of Experimental Zoology* 246:132-138.
- Packard, M. J., Thompson, M. B., Goldie, K. N. and M. Vos. 1988. Aspects of shell formation in eggs of tuatara, *Sphenodon punctatus*. *Journal of Morphology* 197:147-157.
- Searle, S. R. 1987. *Linear Models for Unbalanced Data*. John Wiley and Sons, New York.
- Simkiss, K. 1967. *Calcium in Reproductive Physiology*. Chapman and Hall, London.
- Sinervo, B. and S. C. Adolph. 1989. Thermal sensitivity of growth rate in hatchling *Sceloporus* lizards: environmental, behavioural and genetic aspects. *Oecologia* 78:411-419.
- Thompson, M. B. 1990. Incubation of eggs of tuatara, *Sphenodon punctatus*. *Journal of Zoology, London* 222:303-318.

-
- Thompson, M. B., Newman, D. G. and P. R. Watson. 1991. Use of oxytocin in obtaining eggs from tuatara (*Sphenodon punctatus*). *Journal of Herpetology* 25:101-104.
- Thompson, M. B., Packard, G. C., Packard, M. J. and B. Rose. 1996. Analysis of the nest environment of tuatara, *Sphenodon punctatus*. *Journal of Zoology, London* 238:239-251.
- Thompson, M. B., Stewart, J. R. and B. R. Speake. 2000. Comparison of nutrient transport across the placenta of lizards differing in placental complexity. *Comparative Biochemistry and Physiology* 127A:469-479.
- Tsuji, J., Huey, R. B., van Berkum, F. H., Garland, T. Jr. and R. Shaw. 1989. Locomotor performance of hatchling fence lizards (*Sceloporus occidentalis*): quantitative genetics and morphometric correlates. *Evolutionary Ecology* 3:240-252.
- Tyrrell, C. L., Cree, A. and D. R. Towns. 2000. Variation in reproduction and condition of northern tuatara (*Sphenodon punctatus punctatus*) in the presence and absence of kiore. Science for Conservation No. 153. Department of Conservation, Wellington, New Zealand.
- Wallis, I. R., Henen, B. T. and K. A. Nagy. 1999. Egg size and annual egg production by female desert tortoises (*Gopherus agassizii*): the importance of food abundance, body size, and date of shelling. *Journal of Herpetology* 33:394-408.
- Webb, P. V. 1986. Locomotion and predator-prey relationships. Pp. 24-41. In Feder, M. E. and G. V. Lauder (Eds.). *Predator-prey Relationships: Perspectives and Approaches from the Study of Lower Vertebrates*. University of Chicago Press, Chicago.
- Wilgenbusch, J. C. and D. T. Gantz. 2000. The effects of hormonally induced oviposition on egg viability in the common snapping turtle *Chelydra serpentina*. *Herpetologica* 56:1-7.

Chapter 3. Male biased sex ratio among hatchlings from natural tuatara nests

Nicola J. Nelson¹, Michael B. Thompson², Susan N. Keall¹,

Bryan M. Gartrell³ and Charles H. Daugherty¹

¹ School of Biological Sciences, Victoria University of Wellington, P. O. Box 600, Wellington, New Zealand; ² School of Biological Sciences and Wildlife Research Institute, Heydon-Laurence Building (A08), University of Sydney, NSW 2006, Australia; ³ Karori Veterinary Clinic, 20 Parkvale Road, Karori, Wellington, New Zealand.

3.1. Abstract

Tuatara (*Sphenodon punctatus*) are New Zealand reptiles with temperature-dependent sex determination (TSD) when incubated at constant temperatures. In nature, the sex ratio of hatchlings is a critical demographic parameter, particularly in species with TSD. We investigated the occurrence of TSD in natural nests on Stephens Island by recording natural nest characteristics, including temperature, and ascertaining the gonadal sex of all juveniles from those nests using laparoscopy. Twenty-five nests were selected to encompass the diversity of nest characteristics. Most nests had a northeasterly aspect, and mean depth from the top egg to the soil surface was 103 mm. Eggs were laid in clusters of between one and three layers; mean clutch size was 8.8 eggs. Mean number of days for incubation was 365, and hatching success was 65%. Incubation temperatures throughout the year ranged from 2.9 to 34.4°C, with daily fluctuations of between 0.5 and 15.6°C in summer (December to March). The overall proportion of male hatchlings was 0.64, and sex ratios varied significantly among nests. Sex ratios of nests were correlated with incubation temperature and nest depth, indicating that TSD occurs in nature. Male biased sex ratios are rare in hatchling reptiles. This study represents data from only one nesting season, and air temperatures suggest conditions may have been more favourable for production of males during that season. However,

global warming is likely to skew the hatchling sex ratio towards males if female tuatara are unable to select nest sites according to environmental cues.

Keywords: Incubation, laparoscopy, *Sphenodon punctatus*, tuatara, temperature-dependent sex determination.

3.2. Introduction

Temperature-dependent sex determination (TSD), the control of hatchling sex by embryonic incubation temperature, has been documented in all four extant orders of reptiles (Ewert et al. 1994, Lang and Andrews 1994, Viets et al. 1994, Cree et al. 1995). In tuatara (*Sphenodon punctatus*), for example, eggs incubated artificially at 22°C result in predominantly male hatchlings, while those incubated at cooler temperatures result in predominantly female hatchlings (Cree et al. 1995). Less than 1°C separates male- and female-producing temperatures (Chapter 4).

As the sex ratio of hatchlings of species with TSD varies with the thermal environment (Janzen 1994), global warming may lead to shifts in functional sex ratios (Frazer et al. 1993). Stochasticity of sex ratios could lead to skewed sex ratios in small populations and produce an Allee effect, that is, increased risk of extinction of the population due to inverse density-dependent effects at low numbers (Stephens and Sutherland 1999). Thus, sex ratios are critical parameters that affect population dynamics and can affect population persistence (Gibbons et al. 2000). Knowledge of the effect of TSD on sex ratios in nature is important for species conservation.

Artificial incubation of tuatara eggs has been conducted primarily at constant temperatures (although see Cree et al. 1991a), which bear little resemblance to the variable thermal conditions experienced by natural nests on both a diurnal and seasonal basis (Thompson et al. 1996). Although the natural nest environment of tuatara has been described by Thompson et al. (1996), the hatchlings produced from that study were not sexed. Consequently, the occurrence of TSD in nature was not confirmed for tuatara. If TSD does occur in nature, then the sex ratio of hatchlings might vary between nests, rookeries and years due to differing thermal climates. Any alterations to thermal properties of nesting areas have the potential to cause fluctuations in the population sex ratio.

Tuatara are New Zealand reptiles that are restricted to off-shore islands. They lay about nine flexible-shelled eggs in shallow nests of up to three layers (Packard et al. 1988, Thompson et al. 1996). Females nest every 2-5 years, and incubation takes on average one year to complete (Dendy 1899, Cree et al. 1991b, Thompson et al. 1996). The nesting season on Stephens Island, the location of the largest population of tuatara (Newman 1982), occurs throughout November and December (early summer) when nesting females congregate in exposed rookeries (Thompson et al. 1996).

Stephens Island, a 150 ha Nature Reserve, was historically forested to the cliff edges, restricting nesting tuatara to unvegetated areas on the cliff edge or to temporary gaps in the forest (Dendy 1899, Thilenius 1899). Tuatara nests are unsuccessful under full forest cover, as temperatures do not get high enough for embryonic development (Cree et al. 1989, Thompson et al. 1996). As a result of construction of a lighthouse in 1894-95, much of the forest was cleared and the land subsequently maintained in pasture for

livestock (Brown 2000). An increase in the amount of exposed areas for nesting is speculated to be the reason for the high number of tuatara occurring on Stephens Island today (30-50,000; Newman 1982, Cree and Thompson 1988, Brown 2000). As part of the ecological restoration plan for Stephens Island, much of the pasture is being replanted with native seedlings (Brown 2000).

We sought to investigate the occurrence of TSD in natural nests of tuatara (*S. punctatus*) on Stephens Island by describing natural nest characteristics, recording temperatures of natural nests throughout incubation, and ascertaining the gonadal sex of all juveniles from those nests using laparoscopy. We discuss the potential impact of replanting rookeries and of global warming on incubation temperatures, and hence the future sex ratio of hatchling tuatara on Stephens Island.

3.3. Methods

In November 1998, large cleared areas of habitat were systematically searched for tuatara nests on Stephens Island, Cook Strait, New Zealand. Nests were located by monitoring nesting females. Twenty-five nests, from seven of eight known nesting rookeries (Figure 1), were selected to encompass a diversity of nest characteristics including location, nest depth and soil type. The eighth rookery was not sampled due to the fragility of the substrate. Where more than one nest with similar characteristics was identified in a rookery, a representative was randomly chosen. Soil and grass back-fill was removed from each selected nest, and a waterproof Stowaway[®] Tidbit[®] temperature recorder (recording temperature hourly, dimensions: 30 x 41 x 17 mm, Onset Computer Corporation, Massachusetts, USA) was inserted next to the eggs. The back-fill was

carefully replaced, and details of location and nest characteristics were recorded. Nests were permanently marked with a wooden peg, and nest entrances were covered with 200 x 200 mm square of wire mesh (mesh size 10 x 10 mm) to protect them from other nesting females.

The nests were left undisturbed for approximately 11 months. The thermosensitive period for sex determination in reptiles generally lies within the middle third of development (Yntema 1979, Bull and Vogt 1981). Tuatara embryos were assumed to have their sex determined in nests because staging of embryos puts them past the thermosensitive period, at the stage before hatching, prior to winter (~May; Dendy 1899). In October 1999, the nests were excavated, and hatchlings and eggs were collected and taken to Victoria University of Wellington. All eggs were assigned a unique number on the top surface with a soft (4B) graphite pencil, and the orientation of the egg was maintained throughout captive incubation (Thompson 1990). Eggs were weighed to 1 mg on a Sartorius (GmbH Type 1475, Gottengen, Germany) top pan balance on the day of collection and buried in moist vermiculite (approx. -170 kPa) in 2 L plastic containers. In the laboratory, eggs were incubated at 22°C until hatching. Data loggers were removed from nests, placed with the excavated eggs while in transit to the university, and put into the 22°C incubator until the eggs hatched. This provided a complete set of incubation temperatures for each nest throughout incubation.

Downloading of the data from each logger occurred once all eggs had hatched.

Hatchlings were toe-clipped for individual identification, and weighed and measured (snout-vent length (SVL), total length, jaw width and jaw length). They were reared in similar conditions, with 16 individuals per 600 x 700 mm enclosure. Enclosures were furnished with a leaf litter base, tree bark for shelter, and two water dishes. Juveniles

were fed three times per week with similar amounts of crickets, house flies or blow flies per enclosure regardless of juvenile size or hatching date. Enclosures were exposed to daily temperature variations of between approximately 18-22°C and a 12:12 light/dark cycle beginning at 0600 h throughout the year (Duro-test® True-lite® power twist fluorescent tubes, placed 750 mm above the enclosure).

Laparoscopy of internal reproductive organs was conducted on all animals at about 1 year after hatching, as the sex of juvenile tuatara cannot be ascertained externally until they attain approximately 130-160 mm SVL (pers. comm., Alison Cree). The procedure involved swabbing the right lateral surface of the abdomen with 70% ethanol and injecting approximately 0.05 ml of local anaesthetic (2% lignocaine hydrochloride; Lopaine, Ethical Agents Ltd, Auckland, New Zealand; 27 gauge, ½ inch needle) into the abdominal musculature of each animal, or subcutaneously if juveniles were less than 7 g body mass. A small (2 mm) incision was made through the abdomen wall at the site of injection, approximately one-third anterior from the rear legs. Reproductive organs (gonads and reproductive ducts, where visible) on the right side of the juvenile were examined using a 1.7 mm diameter endoscope with 10x magnification (Olympus A7002, Japan), and gonadal sex ascertained. The incision was sutured with absorbable chromic cat gut (Braun, Germany), and the area dusted with antibiotic powder (Tricin, Jurox, Auckland, New Zealand). Each operation took approximately 5 minutes. All equipment and consumables that were not already sterile were soaked in a non-selective, biodegradable antiseptic (1:200 dilution, Aviscrub, Vetafarm, Waggawagga, Australia).

Figure 1. Stephens Island, Cook Strait, New Zealand showing tuatara nesting rookeries, vegetation and topography.

3.3.1. *Statistical Analyses*

Logistic regression models were developed using SYSTAT 10.0 statistical program to investigate the effects of depth and aspect of nests, position (layer) of eggs within nests (representing the probable thermal gradient within the nest), nest temperature, and combinations of these factors on the sex of juvenile tuatara. Hierarchical models (where variables were added in a stepwise fashion) were compared using chi-square tests ($p < 0.05$) with the test statistic equal to the difference in residual deviance between models and the degrees of freedom equal to the difference in the number of parameters between models. As nest temperature varied on a daily and seasonal basis, four representative values were selected for each nest: minimum, mean, and maximum temperature of nests during summer (Dec-Mar), and mean constant temperature equivalent (cte) in February.

Mean cte was included as a temperature variable as it accounts for the variability of a nest as well as its mean temperature. The cte is defined as the temperature above which half of embryonic development occurs (Georges 1989, Georges et al. 1994). For tuatara, males are predicted to be produced if the cte of a nest exceeds the pivotal temperature for sex determination in artificial conditions; females are predicted to be produced from nests where the cte is lower than the pivotal. For each nest, mean cte during February was calculated using Georges' model (Georges 1989, Georges et al. 1994) with the following inputs: estimated developmental zero equal to 11.1°C (using incubation durations at constant incubation temperatures (Chapter 4 (4.4.1)), and assuming a linear relationship below 18°C), reference temperature equal to 18°C with an incubation period of 264 days, pivotal temperature estimated as 21°C, and hourly temperature records for each nest during February. Temperatures during February were

selected as February was assumed to be within the thermosensitive period for sex determination.

The occurrence of TSD in tuatara in nature was investigated by comparing sex ratios of hatchlings from nests with expected values using chi-square tests. We examined two different hypotheses. The first hypothesis was that the sex ratio of individual nests was 0.5 (proportion of males); the alternative to this hypothesis was that sex ratios of nests differed from 0.5. Failure to reject the null model would imply there was either no TSD in nature, or that in one year the overall ratio was 0.5, or that the sample size was not large enough to detect departure from 0.5. Expected values were derived for each nest based on the number of hatchlings in that nest divided by two. Only 17 of 25 nests sampled were included in these analyses as four nests contained only one hatchling of known sex, three nests were unsuccessful, and one nest not relocated. Degrees of freedom for this test were equal to the number of nests ($n = 17$).

The second hypothesis was that the sex ratio was similar in each nest, but not necessarily 0.5. The alternative hypothesis for this test was that at least one nest had a different sex ratio. Failure to reject the null model would imply there was either no TSD in nature, or that data were insufficient, or temperatures were too alike to demonstrate TSD. The expected values for this test were derived from a standard chi-square table. Degrees of freedom for this test were equal to the difference in the number of parameters between the null hypothesis ($n = 17$) and the alternative hypothesis ($n = 1$), that is, 16. If the overall sex ratio was significantly different from 0.5 (test 1), or sex ratios were not the same over all nests (test 2), then there was evidence to support the occurrence of TSD in nature. We were not able to sex embryos

in the majority of eggs that died during incubation because their contents were either destroyed by insect larvae or embryos were not developed enough. Sex biased mortality is addressed in the discussion.

3.4. Results

3.4.1. Natural Nest Characteristics, Incubation Duration, and Hatching

Success

Eggs were laid between 1 and 15 November 1998, and most nests (18 out of 25) were built with a north-easterly aspect (20 - 60°). Eggs and hatchlings were excavated on 8 and 9 October 1999; one nest could not be relocated. Mean depth to the top egg from the soil surface was 103 mm (range 40 – 200 mm; $n = 24$), and eggs were laid in a cluster of between one and three layers. Mean clutch size was 8.2 eggs (range 3 – 13); total number of eggs was 221 (Table 1). Eggs took between 331 and 400 days to hatch. These figures and the mean number of days to hatching (365) are not representative of what actually occurs in nature as eggs from four nests had hatched before they were relocated (at about 331 days incubation), and after nest relocation all unhatched eggs were incubated at constant temperature until hatching. The nest relocation date was used as a surrogate hatch date for early hatching nests, and this produces an overestimate of hatching date. The duration of incubation of late hatching nests is likely to be an underestimate, as constant 22°C incubation is warmer than they would have experienced in natural nests during October. Incubation in natural nests can take up to 16 months in variable conditions (~ 480 days; McIntyre 1997). All hatchlings and viable eggs ($n = 149$) were retrieved from the 24 relocated nests. Hatching success

overall was 65%, but individual nest success varied greatly. Five nests had 100% hatching success and three nests had 0% hatching success (Table 1).

3.4.2. Temperature

Incubation temperatures were successfully recorded in 23 of the 24 nests recovered; one data logger failed early in incubation. Over the entire year, nest temperatures ranged from 2.9 – 34.4°C. The two nests that experienced these extremes were successful (2.9°C nest minimum, 80% hatching success; 34.4°C nest maximum, 100% hatching success). Nests experienced daily and seasonal fluctuations in temperature to varying degrees. Daily fluctuations ranged from 0.5-15.6°C between December and March. Nest temperatures fluctuated less during winter months.

3.4.3. Sex Ratio

The sex of hatchlings from 21 nests was investigated by laparoscopic examination of gonads ($n = 138$ hatchlings plus 2 embryos). The sex of six additional hatchlings could not be determined due to difficulties locating gonads during the operations ($n = 3$), or uncertainties over identification of gonad morphology ($n = 3$) within the time period set down for operations (time restrictions were necessary to prevent juveniles from overheating). The overall sex ratio was 90 males: 50 females (hereafter sex ratio will be referred to as proportion of males, 0.64). Ten nests produced 100% male hatchlings, four nests produced 100% female hatchlings (Figure 2), and seven nests produced mixed sex ratios ranging from 0.11-0.88 males (Table 1). Only two of the latter nests had a sex ratio of 0.5. Overall, the sex ratio of nests was significantly different from 0.5 ($\chi^2_{(17)} = 102.58, p < 0.001$), and sex ratios varied significantly among nests ($\chi^2_{(16)} =$

80.78, $p < 0.001$). Of the seven rookeries in which the 24 marked nests were located, two rookeries produced only males, two rookeries produced male biased nests, two rookeries produced nests with 0.5 sex ratios, and all nests in one rookery were unsuccessful.

Table 1. Clutch size, hatching success and sex ratios of naturally incubated tuatara nests.

Nest	Clutch size	No. eggs failed in nest	No. hatchlings	No. juveniles sexed	Sex ratio (M:F)
1	11	4	7	7	7:0
2	6	0	4	6* @	6:0
3	11	0	11	10	5:5
4	8	1	7	7	^α 7:0
5	13	0	13	12	12:0
6	Nest not relocated	-	-	-	-
7	10	1	8	8	1:7
8	9	0	9	9	9:0
9	12	4	8	7	7:0
10	10	2	8	8	7:1
11	3	2	1	1	1:0
12	4	1	1	1	0:1
13	9	8	1	1	1:0
14	9	0	9	9	1:8
15	9	8	1	1	1:0
16	11	11	0	-	-
17	10	2	8	8	7:1
18	7	1	6	6	0:6
19	13	6	7	7	3:4
20	8	0	8	8	8:0
21	11	11	0	-	-
22	11	4	7	7	0:7
23	11	1	10	9	0:9
24	11	1	10	8	7:1
25	4	4	0	-	-
Total	221	72 #	144 *	140 @	90:50

Of the 72 eggs that died during incubation in nests, 34 suffered beetle damage, 16 showed no embryonic development, 7 died at early developmental stages, 10 had mid to late stage embryos, and 2 eggs were rotten and could not be assigned to any of these categories. Three eggs had possibly hatched and juveniles had escaped. *Embryos from 5 eggs died after excavation of nests but prior to hatching (2 were punctured during excavation; 3 died during subsequent incubation). @ 138 live juveniles were sexed; 6 juveniles could not be sexed; the embryos from 2 punctured eggs were sexed. ^α Temperature data logger failed in this nest.

Figure 2. The proportion of male tuatara hatchlings in natural nests on Stephens Island.

3.4.4. Temperature and Sex Ratio

Of the four nest temperature estimators, maximum temperature ($p < 0.00004$) and cte ($p < 0.00002$) were the strongest predictors of the sex of hatchlings, probably because they better represented the variation in nest temperatures. Mean temperatures are known to be poor predictors of hatchling sex ratios when nest temperatures fluctuate (Georges 1989). Nest depth also had a significant effect on sex. If temperature was excluded from the analysis, then depth and position of the egg in the nest, and an interaction between these effects, influenced sex, indicating that there is a temperature gradient in the nest mediated by depth (see also Thompson et al. 1996). Males were produced from warm nests, females from cool nests, and mixed sex ratios from intermediate temperatures (Figure 3). Thus, tuatara incubated in natural nests have TSD.

Figure 3. The effect of temperature on sex of hatchlings demonstrating TSD exists in natural nests. The model used to calculate constant temperature equivalents (Georges 1989; Georges et al. 1994) for each nest assumes that hatchling sex depends on the proportion of embryonic development that occurs above the pivotal temperature, not the proportion of time spent above the pivotal temperature.

3.5. Discussion

In species with TSD, biased sex ratios in natural nests are expected and frequently observed (Charnov 1982). The hatchling sex ratios reported for populations of reptiles with TSD predominantly range from 0.5 (proportion of males) to less than 0.5, that is, greater proportions of females (Tokunaga 1984, Bull and Charnov 1988, 1989, Ewert and Nelson 1991, but see Thorbjarnarson 1997, Lance et al. 2000). For example, the loggerhead sea turtle (*Caretta caretta*) has a hatchling sex ratio of 0.1 at rookeries in Florida, where 90% of North American clutches are laid (Mrosovsky and Provancha 1989, 1992); Brazilian beaches produce hatchling sex ratios of close to 0.2 (Marcovaldi et al. 1997). Hatchling sex ratios of alligators are 0.2, while freshwater crocodiles are 0.35 (reviewed in Bull and Charnov 1989). Hatchling sex ratios of greater than 0.5 for reptiles with TSD are rare (e.g., Bull and Charnov 1989, Ewert and Nelson 1991,

Thorbjarnarson 1997, Vogt and Bull 1984), but are predicted for theoretical reasons (Charnov and Bull 1989).

TSD occurs in natural nests of tuatara; nest construction in 1998 resulted in a male biased sex ratio of 0.64 over the nests examined. Possible explanations for a male biased sex ratio from a single nesting season include a bias from population sampling (i.e., in 1998 more females laid their eggs in male producing nests just by chance alone), experimental sampling (i.e., our selection of nest sites resulted in non-random selection of nests that had high temperatures), a bias caused by sampling in one year only (e.g., if 1998 was a particularly warm year), or sex-biased mortality. Alternatively, our sample may be representative of the incubation temperatures normally experienced on Stephens Island, suggesting that the majority of nests on this island exhibit high (male producing) incubation temperatures in a majority of years. We selected nests on a stratified random basis to capture the variability between and within rookeries, thus attempting to sample representative nests from the whole population.

We cannot speculate on whether individual female tuatara nested in warmer sites in our sampling year, but we do know that rookeries were located in similar positions to those sampled by Thompson et al. in 1986 (Thompson et al. 1996). Tuatara eggs are laid in exposed locations in relatively shallow nests, resulting in temperature gradients within nests (Thompson et al. 1996), by which mixed sex ratio nests are sometimes produced. Temperature fluctuations between years could explain a biased sex ratio in any one year. During the summer of 1998/99 when sex was likely to be determined in embryos in the marked nests, air temperatures (minimum, mean and maximum daily averages) were higher than the averages for years 1990 to 2001. For example, mean air

temperature in January 1999 was 17.2 °C versus between 15.2 and 16.5°C for January 1990-98 and 2000-01. Artificial incubation experiments demonstrate that the pivotal constant temperature for *S. punctatus* from Stephens Island is likely to be between 21 and 22°C, and that less than 1°C separates male and female producing temperatures (Chapter 4). Thus, it seems possible that even minor fluctuations in temperatures in natural conditions may alter sex ratios and that the 1998/99 summer was more favourable for production of males, and preceding and following years could have produced more females.

High density nesting areas are usually chosen for examination of sex ratios in hatchling reptiles, and these are also likely to be areas of higher quality and hatching success (Reinhold 1998). However, embryos incubated in these areas are more likely to differentiate as females for species with nest site philopatry (Reinhold 1998). Hence, collections from such areas may not reflect population-wide sex ratios in all instances (Bull and Charnov 1989). Although we do not know whether female tuatara are philopatric, or even if individuals nest on the same rookery in subsequent nesting seasons, bias through sampling rookeries with high nesting densities cannot account for the male-biased sex ratio we observed. We chose nests in high density nesting areas (rookeries) because very few nests were located elsewhere. Hatching success was high in these areas, but this was partly as a result of the nest protection provided in this study. No female biased sex ratio was achieved on any rookery, and only on two rookeries did the sex ratio get as low as 0.5.

Differential mortality as a function of incubation temperature has been reported in pine snakes, but death of embryos occurred late in development (Burger and Zappalorti

1988). We discount egg mortality as a cause for the bias in hatchling sex ratio. Insect larvae destroyed half of the unsuccessful eggs. In nests where some eggs were destroyed by beetle larvae, other eggs hatched successfully. We cannot be sure whether insect larvae were the cause of death or ate eggs containing embryos that had already died. If insect larvae do kill embryos, then we assume this would be independent of sex. If insect larvae eat eggs that have already died, then this could occur any time between laying and excavation of nests when most eggs had still not hatched. We have evidence of only ten embryos that died at a stage late enough for sex determination to have occurred. Even if these were all females, then the sex ratio would still be significantly different from 0.5.

A 0.5 sex ratio after hatching is characteristically required for an evolutionarily stable sex allocation strategy (Fisher 1930, Reinhold 1998). One season of data may not be representative of the hatchling sex ratio over the long-term, as tuatara live to at least 60 years (Castanet et al. 1988). Also, as tuatara take about 13 years to reach sexual maturity (Dawbin 1982, Castanet et al. 1988), hatchling sex ratios may not represent those at sexual maturity. In tuatara, an operational sex ratio with a larger proportion of females might be expected, as female tuatara reproduce only about every four years (Cree et al. 1991b). Nothing is known of whether juvenile mortality is differential based on sex, or of the sex ratio of the adult population on Stephens Island.

Estimates for temperature increases in the next 100 years, as a result of global warming, are between 1.4 and 5.8°C (IPCC 2001). Although 1998/99 incubation temperatures, which produced a predominance of males, may not be representative of the past decade, they provide insight into the effect further warming could have on the sex ratio. If

female tuatara continue to use the same rookeries and construct nests with the characteristics described in 1986 (Thompson et al. 1996) and 1998 (the present study), then global warming is likely to skew the population sex ratio toward more males.

Another influence on the nest environment of Stephens Island is revegetation of sheep-grazed pastures as part of ecological restoration. Early stages of revegetation are likely to reduce soil temperatures directly below plantings due to shading. If tuatara still choose to nest in the currently known rookeries, then revegetation could possibly result in an increase in the proportion of females produced at these sites. However in the longer term, nesting is unlikely to be successful at replanted sites, as tuatara do not build nests in forest, presumably because temperatures do not get high enough for embryonic development to occur (Cree et al. 1989, Thompson et al. 1996).

TSD does occur in natural nests of tuatara, and variation in the thermal environment has the potential to vary the hatchling sex ratio and ultimately the population sex ratio. If female tuatara are not able to use environmental cues for nest site selection and construction, then global warming is likely to skew the population sex ratio towards a predominance of males. More data are required to evaluate variation in hatchling sex ratios over several seasons and its effect on the population sex ratio.

3.6. References

- Brown, D. 2000. Stephens Island: Ark of the Light. Derek Brown, Havelock, New Zealand.
- Bull, J. J. and E. L. Charnov. 1988. How fundamental are Fisherian sex ratios? *Oxford Survey of Evolutionary Biology* 5:96-135.
- Bull, J. J. and E. L. Charnov. 1989. Enigmatic reptilian sex ratios. *Evolution* 43:1561-1566.
- Bull, J. J. and R. C. Vogt. 1981. Temperature sensitive periods of sex determination in Emydid turtles. *Journal of Experimental Zoology* 218:435-440.
- Burger, J. and R. T. Zappalorti. 1988. Effects of incubation temperature on sex-ratios in pine snakes: differential vulnerability of males and females. *The American Naturalist* 132:492-505.
- Castanet, J., Newman, D. G. and H. Saint-Girons. 1988. Skeletochronological data on the growth, age, and population structure of the tuatara, *Sphenodon punctatus*, on Stephens and Lady Alice Islands, New Zealand. *Herpetologica* 44:25-37.
- Charnov, E. L. 1982. The Theory of Sex Allocation. Princeton University Press, Princeton.
- Cree, A. and D. Butler. 1993. Tuatara Recovery Plan (*Sphenodon* spp.). Threatened Species Recovery Plan Series No. 9. Department of Conservation, Wellington, New Zealand.
- Cree, A., Hay, J. M., Daugherty, C. H. and S. N. Keall. 1991a. Effects of constant and variable incubation temperatures on growth of tuatara (*Sphenodon guntheri*) embryos. *Proceedings of the Physiological Society of New Zealand* 11:59 (abstract).
- Cree, A., Cockrem, J. F., Brown, M. A., Watson, P. R., Guillette, L. J. Jr., Newman, D. G. and G. K. Chambers. 1991b. Laparoscopy, radiography, and blood samples as techniques for identifying the reproductive condition of female tuatara. *Herpetologica* 47:238-249.
- Cree, A. and M. B. Thompson. 1988. Unravelling the mysteries of tuatara reproduction. *Forest and Bird* 250:14-16.
- Cree, A., Thompson, M. B. and C. H. Daugherty. 1995. Tuatara sex determination. *Nature* 375:543.
- Cree, A., Thompson, M. B., Guillette, L. J. Jr., Hay, J. M. and M. E. McIntyre. 1989. Embryonic development of tuatara in forested and open habitats on Stephens Island, New Zealand. *New Zealand Journal of Zoology* 16:270.

- Dawbin, W. H. 1982. The tuatara *Sphenodon punctatus*: aspects of life history, growth and longevity. Pp. 237-250. In Newman, D. G. (Ed.). New Zealand Herpetology. New Zealand Wildlife Service Occasional Publication No. 2.
- Dendy, A. 1899. Outlines of the development of the tuatara, *Sphenodon (Hatteria) punctatus*. *Quarterly Journal of the Microscopical Society* 42:1-87.
- Ewert, M. A. and C. E. Nelson. 1991. Sex determination in turtles: diverse patterns and some possible adaptive values. *Copeia* 1991:50-59.
- Ewert, M. A., Jackson, D. R. and C. E. Nelson. 1994. Patterns of temperature-dependent sex determination in turtles. *Journal of Experimental Zoology* 270:3-15.
- Fisher, R. A. 1930. The Genetic Theory of Selection. Clarendon Press, Oxford.
- Frazer, N. B., Green, J. L. and J. W. Gibbons. 1993. Temporal variation in growth rate and age at maturity of male painted turtles, *Chrysemys picta*. *American Midlands Naturalist* 130:314-324.
- Georges, A. 1989. Female turtles from hot nests: is it duration of incubation or proportion of development at high temperatures that matters? *Oecologia* 81:323-328.
- Georges, A., Limpus, C. and R. Stoutjesdijk. 1994. Hatchling sex in the marine turtle *Caretta caretta* is determined by proportion of development at a temperature, not daily duration of exposure. *Journal of Experimental Zoology* 270:432-444.
- Gibbons, J. W., Scott, D. E., Ryan, T. J., Buhlmann, K. A., Tuberville, T. D., Metts, B. S., Greene, J. L., Mills, T., Leiden, Y., Poppy, S. and C. T. Winne. 2000. The global decline of reptiles, déjà vu amphibians. *BioScience* 50:653-666.
- IPCC. 2001. Climate Change 2001: The Scientific Basis. Inter-governmental Panel on Climate Change. Cambridge University Press, Cambridge.
- Janzen, F. J. 1994. Climate change and temperature-dependent sex determination in reptiles. *Proceedings of the Natural Academy of Science* 91:7487-7490.
- Lance, V. A., Elsey, R. M. and J. W. Lang. 2000. Sex ratios of American alligators (Crocodylidae): male or female biased? *Journal of Zoology, London* 252:71-78.
- Lang, J. W. and H. V. Andrews. 1994. Temperature-dependent sex determination in crocodilians. *Journal of Experimental Zoology* 270:28-44.
- Marcovaldi, M. A., Godfrey, M. H. and N. Mrosovsky. 1997. Estimating sex ratios of loggerhead turtles in Brazil from pivotal incubation durations. *Canadian Journal of Zoology* 75:755-770.
- McIntyre, M. 1997. Conservation of the Tuatara. Victoria University Press, Wellington, New Zealand.

-
- Mrosovsky, N. and J. Provancha. 1989. Sex ratio of loggerhead sea turtles hatching on a Florida beach. *Canadian Journal of Zoology* 67:2533-2539.
- Mrosovsky, N. and J. Provancha. 1992. Sex ratio of hatchling loggerhead sea turtles: data and estimates from a 5-year study. *Canadian Journal of Zoology* 70:530-538.
- Newman, D. G. 1982. Tuatara, *Sphenodon punctatus*, and burrows, Stephens Island. In Newman D. G. (Ed.). New Zealand Herpetology. Occasional Publication No. 2. New Zealand Wildlife Service, Department of Internal Affairs, Wellington, New Zealand.
- Packard, M. J., Thompson, M. B., Goldie, K. N. and M. Vos. 1988. Aspects of shell formation in eggs of the tuatara, *Sphenodon punctatus*. *Journal of Morphology* 197:147-157.
- Reinhold, K. 1998. Nest-site philopatry and selection for environmental sex determination. *Evolutionary Ecology* 12:245-250.
- Stephens, P. A. and W. J. Sutherland. 1999. Consequences of the Allee effect for behaviour, ecology and conservation. *Trends in Ecology and Evolution* 14:401-405.
- Thilenius, G. 1899. Vorläufiger bericht über die eiablage und erste entwicklung der *Hatteria punctata*. *Sitzungsberichte der Königlich Preussischen Akademie der Wissenschaften zu Berlin* 1899:247-256.
- Thompson, M. B. 1990. Incubation of eggs of tuatara, *Sphenodon punctatus*. *Journal of Zoology, London* 222:303-318.
- Thompson, M. B., Packard, G. C., Packard, M. J. and B. Rose. 1996. Analysis of the nest environment of tuatara *Sphenodon punctatus*. *Journal of Zoology, London* 238:239-251.
- Thorbjarnarson, J. 1997. Are crocodilian sex ratios female biased? The data are equivocal. *Copeia* 1997:451-455.
- Tokunaga, S. 1984. Morphological variation and sexual dimorphism in *Gekko japonicus* from Fukuoka, Northern Kyushu, Japan. *Japanese Journal of Herpetology* 10:80-88.
- Viets, B. E., Ewert, M. A., Talent, L. G. and C. E. Nelson. 1994. Sex determining mechanisms in squamate reptiles. *Journal of Experimental Zoology* 270:45-56.
- Vogt, R. C. and J. J. Bull. 1984. Ecology of hatchling sex ratio in map turtles. *Ecology* 65:582-587.
- Yntema, C. L. 1979. Temperature levels and period of sex determination during incubation of eggs of *Chelydra serpentina*. *Journal of Morphology* 159:17-28.

Chapter 4. **Egg mass governs hatchling size, but incubation regime influences post-hatching growth of tuatara**

Nicola J. Nelson¹, Michael B. Thompson², Susan N. Keall¹ and Charles H. Daugherty¹

¹School of Biological Sciences, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand; ²School of Biological Sciences and Wildlife Research Institute, Heydon-Laurence Building (A08), University of Sydney, NSW 2006, Australia.

4.1. Abstract

Incubation temperature is known to affect many aspects of reptile hatchling phenotype. Hatchling size is a phenotypic trait judged likely to influence fitness. The prevailing view is that hatchling size is an indicator of quality. We investigate whether incubation temperature affects size of tuatara hatchlings, and if so, whether the influence is still apparent at ten months post-hatching. We find that incubation temperature does not greatly influence size of hatchlings. Incubation temperature significantly influences size by the time artificially incubated tuatara reach ten months of age, despite similar rearing conditions post-hatching. Parental effects predominantly influence size of hatchling tuatara, and they are still important at ten months of age. Initial egg mass is the most important factor affecting size of hatchlings from artificial incubation regimes. Artificial incubation at constant temperatures is used to produce founders for new captive and wild populations of tuatara (*Sphenodon punctatus*) and to augment existing rare populations. We compare size of artificially incubated hatchlings with those incubated in natural nests, during the same season. Constant artificial incubation conditions result in larger juveniles than those from wild incubation by ten months of age, but they appear to be less aggressive than those from more variable natural incubation conditions. It is not known whether the “bigger is better” hypothesis applies

to tuatara, but if so, artificial incubation is a good method for producing founders for new populations. Evidence from size patterns of tuatara incubated in natural nests supports differential fitness models for the adaptive significance of temperature-dependent sex determination.

Keywords: *Sphenodon punctatus*, tuatara, incubation, size, maternal influence, post-hatching growth.

4.2. Introduction

Theoretical models for the evolution of temperature-dependent sex determination (TSD) in reptiles predominantly revolve around the following assumptions: that incubation temperature affects hatchling phenotype, that hatchling traits reflect individual fitness, and that one or both of these relationships differ between the sexes (Charnov and Bull 1977, Burger and Zappalorti 1988, Rhen and Lang 1995, Shine et al. 1995, Tousignant and Crews 1995, Janzen 1996, Shine 1999). Incubation temperature is known to affect many aspects of reptile hatchling phenotype, including size, shape, colour, sex, performance traits, metabolism, growth rates and survival (Vinegar 1974, Bull 1980, Ferguson and Joanen 1982, Gutzke and Packard 1987, Joanen et al. 1987, Burger and Zappalorti 1988, Packard and Packard 1988, Burger 1989, 1990, Brooks et al. 1991, Deeming and Ferguson 1991, Van Damme et al. 1992, Spotila et al. 1994, Cree et al. 1995, Tousignant and Crews 1995, Shine and Harlow 1996, Elphick and Shine 1998, Steyermark and Spotila 2000, 2001).

Few reptile studies have assessed persistence of incubation effects past hatching, let alone throughout ontogeny, or the biological significance of such effects (Elphick and Shine 1998, Shine 1999). Traits judged likely to influence fitness include hatchling size, shape, activity, performance, thermoregulatory preferences, and time of hatching (Shine 1983, Ferguson and Fox 1984, Shine and Harlow 1996, Elphick and Shine 1998, O'Steen 1998). For example, the prevailing view is that hatchling size is an indicator of quality (Head et al. 1987, Miller et al. 1987, Packard and Packard 1988). In the short term, larger juvenile side-blotched lizards (*Uta stansburiana*), for example, are more likely to survive, and this has been attributed to competitive advantages in food acquisition and reduced vulnerability to predation than smaller conspecifics (Ferguson and Fox 1984). In the longer term, the "bigger is better" hypothesis implies that smaller male reptiles may not be as successful at maintaining territories, attracting females, or achieving mating opportunities (Froese and Burghardt 1974, Swingland and Coe 1979, Berry and Shine 1980, Webb et al. 1987, Lang et al. 1989). However, not all studies of reptiles support the view that large size conveys an advantage over smaller similar aged conspecifics (Congdon et al. 1999).

Analysis of incubation effects on phenotype, persistence of such effects, and their relevance for fitness are complicated for several reasons. Most studies of influences on hatchling phenotypes have been conducted at constant artificial incubation conditions. The results seem to be broadly applicable to natural incubation conditions (e.g. Shine et al. 1997), but detailed comparisons of hatchling phenotypes from artificial incubation conditions with those produced under natural incubation regimes are lacking. Hence, artificial incubation is often used as a tool for conservation of reptiles without assessment of the quality of individuals produced. Also, characteristically, incubation

effects are correlated with differences due to sex resulting from the very nature of TSD (but see Rhen and Lang 1995). Other influences on hatchling phenotype include maternal choice of oviposition site and timing (which affect nest temperatures), variable allocation of nutrients to eggs, and paternal and maternal genetic aspects (Schwarzkopf and Brooks 1987, Cagle et al. 1993, Rhen and Lang 1995, Olsson et al. 1996, Shine and Harlow 1996, Sinervo and Doughty 1996, Reinhold 1998, Steyermark and Spotila 2001). Parental factors are not always included in analyses.

Tuatara (*Sphenodon* spp.) are medium-sized, sexually dimorphic reptiles with TSD (Dawbin 1982, Cree et al. 1995). Now restricted to offshore islands of New Zealand, they are of high conservation importance (Cree and Butler 1993) and are biologically significant as the sole living representatives of the reptilian order Sphenodontia (*sensu* Benton 1990, Cree and Butler 1993). Eggs have been collected and artificially incubated to produce founders for new captive and wild populations and to augment existing rare populations (Nelson 1998, Daugherty 1998). These long-lived animals have low fecundity, do not exhibit secondary sexual characteristics until about 10 years of age and do not reach sexual maturity until they are about 15 years old (Dendy 1899, Dawbin 1982, Cree et al. 1991, Cree 1994, Thompson et al. 1996). Thompson (1990) assessed effects of constant incubation temperatures on tuatara eggs up to the time of hatching, but the biological significance and persistence of effects of incubation environments on fitness and lifetime reproductive success can only be ascertained in the longer term. We investigate whether artificial incubation is a suitable method for producing founders for new populations in the short term by determining whether incubation temperature affects size of tuatara hatchlings, and if so, whether the influence is still apparent at ten months post-hatching. We also compare size of

artificially incubated hatchlings and juveniles with those incubated in natural nests, during the same season. We examine the relationship of hatchling size with incubation temperature and sex, and investigate whether results could theoretically support differential fitness models for the evolution of TSD in tuatara.

4.3. Methods

Tuatara (*S. punctatus*) eggs from a single laying season were incubated in two main treatment groups: at constant temperatures in the laboratory or at variable temperatures in natural nests.

Eggs were collected for artificial incubation from Stephens Island, in the Marlborough Sounds, New Zealand. Between 1-21 November 1998, 320 eggs were collected using two methods: induction of oviposition by injection of synthetic oxytocin (Oxytocin-s, conc. 10 IU/ml, Intervet International BV, Boxmeer, Holland; $n = 166$) and recovery of eggs laid naturally in nests ($n = 154$; Chapter 2). November is within the natural egg-laying season for tuatara on Stephens Island (Thompson 1990, Thompson et al. 1991).

All artificially incubated eggs were assigned a unique number written on the top surface of the egg with a soft (4B) graphite pencil, and the orientation of the egg was maintained upright throughout the study (Thompson 1990). Eggs were weighed to 1 mg on a Sartorius (GmbH Type 1475, Gottengen, Germany) top pan balance on the day of collection and placed half buried in moist vermiculite (approx. -170 kPa) at room temperature on the island (approx. $18-22$ °C). On 21 November 1998, the eggs were flown by helicopter to Victoria University of Wellington for incubation.

Each incubation box comprised a sealed 2 L plastic container half-filled with moistened vermiculite (-170 kPa), and contained four induced and four naturally laid eggs.

Clutches were divided equally into three groups, and each group was randomly assigned to one of three constant incubation temperatures (18, 21 or 22°C). Eggs were then randomly assigned to an incubation box within each treatment. Eggs were weighed weekly, when distilled water was added to incubation boxes to compensate for small losses from the container and uptake by the eggs, thus maintaining a water potential of approximately -170 kPa. Incubators were calibrated prior to use, and incubation temperatures were checked daily. Temperatures were recorded by dataloggers (waterproof Stowaway[®] Tidbit[®] temperature recorders). Incubation boxes were moved within each incubation chamber daily.

During the period when eggs were collected for artificial incubation, twenty-five naturally laid nests were selected and marked for natural incubation (Chapter 3). Selected nests encompassed a diversity of nest characteristics including location, nest depth and soil type. Where more than one nest with similar characteristics was identified in a rookery, a representative was randomly chosen. Soil and grass back-fill was removed from each nest, and a waterproof Stowaway[®] Tidbit[®] temperature recorder (recording temperature hourly, dimensions: 30 x 41 x 17 mm, Onset Computer Corporation, Massachusetts, USA) was inserted next to the eggs. Eggs were not disturbed. The back-fill was carefully replaced, and details of location and nest characteristics were recorded. Nests were permanently marked with a wooden peg, and nest entrances were covered with 200 x 200 mm square of wire mesh (mesh size 10 x 10 mm) to protect them from excavation by other nesting females.

After approximately 11 months, the natural incubation period, the nests were excavated, and hatchlings and eggs were collected and taken to Victoria University of Wellington. All eggs were numbered and placed in incubation boxes as for the artificially incubated eggs, and incubated at 22°C until hatching. Data loggers were removed from nests, placed with the excavated eggs while in transit to the university, and put into the 22°C incubator until the eggs hatched. This provided a complete set of incubation temperatures for each nest throughout incubation. Downloading of the data from each logger occurred once all eggs had hatched.

Hatchlings from all treatments were weighed, measured (snout-vent length (SVL), total length, head width (widest part) and jaw length (snout to posterior end of jaw on right side)), and individually marked with a toe-clip on the day they hatched, and subsequently weighed and measured monthly until 10 months of age (with the exception of juveniles from 21 and 22°C treatments which were not weighed or measured at two months of age). Hatchlings were reared in similar conditions, with 16 individuals per 600 x 700 mm enclosure. Enclosures were furnished with a leaf litter base, tree bark for shelter, and two water dishes. Juveniles were fed three times per week with similar amounts of crickets, house flies or blow flies per enclosure regardless of juvenile size or hatching date. Enclosures were exposed to daily temperature variations of between approximately 18-22°C and a 12:12 light/dark cycle beginning at 0600 h throughout the year (Duro-test[®] True-lite[®] power twist fluorescent tubes, placed 750 mm above the enclosure). Hatching date was controlled for within individual enclosures.

Laparoscopy of internal reproductive organs was conducted at about one year after hatching to ascertain the sex of juveniles (Chapter 3). Juvenile tuatara cannot be sexed externally with confidence until they attain approximately 130-160 mm SVL (pers. comm., Alison Cree).

4.3.1. Statistical Analyses

Multivariate analysis of variance (MANOVA) tests in SAS (SAS Institute) were performed to search for statistically significant factors, covariates and interaction terms ($p < 0.05$) affecting size of juveniles. Artificially and naturally incubated juvenile data sets were analysed separately. Dependent variables included in analyses of artificially incubated young were the following measurements at hatching or ten months of age: jaw length, jaw width, SVL, total length and mass. Fixed factors included incubation temperature or sex. These factors were never included together as incubation temperature co-varies with sex (TSD). Parental factors investigated included clutch as a random effect and initial egg mass as a covariate. We analysed three sets of data from artificially incubated young. The first included all artificially incubated juveniles ($n = 278$ for all measurements except mass at hatching; $n = 277$ for mass at hatching). The second included a subset of female hatchlings, hence controlling for any influence of sex on size. This data set included all juveniles resulting from 18 and 21°C artificial incubation treatments, minus three males produced at 21°C ($n = 170$). The third data set investigated included only females from the 21°C treatment and males from the 22°C treatment, that is, incubation temperature was controlled for as much as possible ($\pm 1^\circ\text{C}$) within the experimental design ($n = 176$ for all measurements except mass at hatching; $n = 175$ for mass at hatching). In this analysis, any influence of incubation temperature can equally be attributed to an influence of sex. Individuals were not included in

analyses if records were incomplete, or eggs could not be matched to a clutch, and it was therefore uncertain whether they represented a whole or partial clutch. This occurred when eggs were opportunistically obtained from the soil surface as opposed to an underground nest.

The size data for naturally incubated young were analysed including sex as a fixed factor, and incubation temperature or clutch identity as random factors. The two random factors were not included together as in most cases clutch identity represented the same information as the temperature variable. We chose the following variables to represent incubation temperatures in natural nests: mean temperature throughout incubation, variance (standard deviation ²) in temperature throughout incubation, duration of incubation, or mean constant temperature equivalent in February (calculated in Chapter 3). Initial egg mass was not available for use as a covariate for naturally incubated young. The complete data set for analysis of the natural incubation regime effects on size included individuals from all nests for which temperature data were obtained that had complete measurement records ($n = 125$ for all measurements at hatching; $n = 124$ for all measurements at ten months).

Analyses of variance and covariance (ANOVA, ANCOVA) using the general linear model (GLM) procedure in SAS were then conducted on each measurement variable respectively using MANOVA results as guides for combinations of factors and covariates. All data sets satisfied assumptions of normality and homogeneity of variances. The few outliers that were identified were retained in the datasets.

Hypothesis test results are not generally reported due to the following reasons: 1. the large number of combinations of factors, covariates, and interactions required to gain a

complete picture of all influences on hatchling size and shape over all measurements, 2. the uncertainty of which groups of analyses to combine for Bonferroni corrections, and hence 3. the high chance of obtaining misleading results. Instead, sums of squares of errors were used to calculate Akaike Information Criteria (AIC; Burnham and Anderson 1998) for each combination or model. We used the small-sample version of the AIC equation (termed AIC_c) designed for cases where there are relatively few data per estimated parameter (Burnham and Anderson 1998). AIC_c s for all models were compared for each dependent variable respectively to evaluate the model that best explained that data set (i.e., had the lowest AIC_c).

We acknowledge a potential problem of pseudo-replication with respect to artificial incubation temperature as only one incubator was used per temperature treatment. This is, however a standard technique for artificial incubation of reptilian eggs. Incubation box effects were not significant and were left out of all analyses. Enclosure effects could not be allowed for due to the limitations imposed on the experimental design by highly variable hatching dates among temperature treatments. Effects of induction on hatchling size were accounted for by including initial egg mass as a covariate in analyses (Chapter 2). Further effects of induction, for example, on incubation duration, were not investigated.

All means of measurements are presented $\pm$ one standard error. All significant factors determined by MANOVA and MANCOVA analyses are underlined ($p < 0.05$).

4.4. Results

Clutch size, egg mass, effects of induction of eggs on hatchling size, and hatching success as a result of artificial incubation are presented in Chapter 2. Incubation

temperatures, nest characteristics, incubation duration, hatching success, and sex ratios of natural nests are presented in Chapter 3.

4.4.1. Duration of Incubation

Incubation took on average 165 ± 0.54 days at constant 22°C ($n = 113$), 183 ± 0.60 days at 21°C ($n = 80$) and 264 ± 2.03 days at 18°C ($n = 105$). At variable temperatures in natural nests, incubation took on average 365 ± 1.92 days ($n = 136$). Note, however, that the majority of naturally incubated eggs finished incubation under artificial constant 22°C , which may have resulted in faster development than would have occurred in natural nests (Figure 1).

Figure 1. Duration of incubation at constant temperatures in captivity (18, 21, and 22°C) and at variable natural temperatures. Temperatures within natural nests ranged between 2.9 and 34.4°C .

4.4.2. Size at Hatching and Ten Months

Mean SVL of hatchling tuatara from all treatments was 53 mm, and individuals varied in mass from 2.803 to 6.001 g. Jaw lengths ranged from 12.1 to 17.8 mm and jaw widths from 8.9 to 12.4 mm. By ten months, all juveniles had increased in size for all measurements. For example, SVL varied from 62 to 95 mm (Table 1).

4.4.3. Effects of Artificial Incubation Temperature on Sex

Incubation temperature determines sex in tuatara (Cree et al. 1995; Table 2). Females were produced at 18°C, predominantly females were produced at 21°C, and males were produced at 22°C. The pivotal temperature lies between 21 and 22°C. These results demonstrate a similar pattern of sex determination compared to earlier studies (Cree et al. 1995), that is females produced at cooler temperatures and males at warmer temperatures, but the expected sex ratios (Chapter 1) vary, probably due to greater sophistication of temperature recording and monitoring techniques, and more reliable equipment in this study.

4.4.4. Effects of Artificial Incubation on Tuatara Size

Duration of incubation

Over all artificial incubation treatments, SVL of hatchlings was not influenced by incubation duration ($F_1 = 0.01$, $p = 0.9142$), despite those embryos incubated at 18°C taking approximately 60% longer to develop than those at 22°C. However, within the 21 and 22°C treatments respectively, eggs that incubated longer produced significantly larger hatchlings ($F_1 = 8.88$, $p = 0.0039$; $F_1 = 48.46$, $p = 0.0001$ respectively; Figure 2). By ten months of age, incubation duration was a significant influence on juvenile SVL ($F_1 = 67.13$, $p = 0.0001$). That is, juveniles that developed over the longest period (at 18°C) were smaller.

Table 1. Summary of measurements of tuatara at hatching and ten months from artificial (18, 21 and 22°C) and variable natural incubation temperatures.

	22°C					21°C					18°C					Nat. nests (variable temperature)				
	Mean	SE	Min.	Max.	n	Mean	SE	Min.	Max.	n	Mean	SE	Min.	Max.	n	Mean	SE	Min.	Max.	n
<i>Jaw Length</i>																				
Hatching	16.4	0.07	13.1	17.8	110	16.2	0.09	12.1	17.6	79	15.9	0.07	14.3	17.1	105	15.7	0.05	13.5	17.4	136
10 months	22.9	0.08	20.9	24.8	110	23.2	0.10	19.5	25.1	79	21.9	0.10	17.9	24.7	105	21.0	0.10	17.2	23.8	135
<i>Jaw Width</i>																				
Hatching	11.2	0.06	8.9	12.4	110	11.4	0.05	10.2	12.4	79	11.3	0.05	10.0	12.2	105	11	0.04	9.4	12.0	136
10 months	16.3	0.08	11.9	18.4	110	16.7	0.08	14.9	18.1	79	15.7	0.08	13.0	17.7	105	15	0.08	12.7	18.8	135
<i>Snout-vent Length</i>																				
Hatching	53	0.23	45	58	110	53	0.25	48	58	79	53	0.25	46	59	105	53	0.18	46	58	136
10 months	84	0.39	64	95	110	86	0.40	77	93	79	80	0.43	64	92	105	76	0.45	62	88	135
<i>Total Length</i>																				
Hatching	107	0.52	90	121	110	108	0.50	95	117	79	105	0.54	89	118	105	106	0.44	92	118	136
10 months	163	1.58	93	199	110	167	1.68	127	192	79	153	1.43	98	192	105	125	1.61	76	175	135
<i>Mass</i>																				
Hatching	4.548	0.058	2.807	5.775	110	4.699	0.069	3.022	5.916	79	4.652	0.062	3.102	5.912	105	4.594	0.056	2.803	6.001	136
10 months	17.654	0.254	6.769	28.483	110	18.083	0.299	12.495	23.780	79	14.053	0.228	6.498	21.466	105	12.25	0.215	5.946	21.302	135

Table 2. Effects of artificial incubation temperature on sex.

Artificial incubation temperature (°C)	No. eggs incubated	No. hatched	Sex (% male)	No. survived to 10 months
18	120	105	0	105
21	80	80	4	79
22	120	113	100	111

Figure 2. Artificial incubation treatment (18, 21 and 22°C) did not affect snout-vent length (SVL) of tuatara hatchlings.

Incubation temperature and parental effects

The primary influence on size of hatchlings incubated artificially and analysed by the MANOVA procedure was parental, that is, initial egg mass or clutch identity.

MANOVA results for all data sets investigated suggested incubation temperature/sex, clutch identity and initial egg mass, and all two- and three-way interactions influenced

size of hatchlings (Table 3 (a)). However, incubation temperature/sex only influenced size of hatchlings through interaction terms with clutch identity or initial egg mass.

At ten months post-hatching, the same global MANOVA model of incubation temperature, clutch identity and initial egg mass, including all two- and three-way interactions, best represented all data sets. However, none of the factors tested were significant influences, except as interaction terms (Table 3 (a)).

Common features of models using the GLM/AIC_c procedure that best described influences on all individual measurements of size respectively were not apparent for the data set including all artificially incubated hatchlings. Incubation temperature, clutch identity and initial egg mass appeared to influence jaw length, snout-vent length, total length and mass; interactions between incubation temperature and initial egg mass influenced jaw length and mass. Hatchlings from 18°C had smaller jaw lengths and total lengths than those from other artificial treatments (Table 1, Figures 3 and 4).

However, the influence of incubation temperature on snout-vent length and mass was not obvious from the data (Table 1, Figures 5 and 6). Jaw width was influenced only by parental factors (Figure 7). At ten months, all measurements were best described by a model including incubation temperature, initial egg mass and their interaction. For example, hatchlings from larger eggs had longer snout-vent lengths and this influence was still apparent at ten months post-hatching (Figure 8). This pattern was more pronounced for juveniles from 18°C than those from 21 or 22°C.

Table 3. Incubation temperature and parental effects on size of artificially incubated tuatara. Influences investigated included incubation temperature (it) or sex, clutch identity (clutch) and egg mass at laying (iem).

(a) Models that best represent size data including jaw length, jaw width, SVL, total length and mass using the MANOVA procedure:

Data set	Best approximating models ²	n	F approximation of Wilks' lambda statistic	P
All juveniles at hatching	<div> it + <u>clutch</u> + <u>iem</u> + <u>it*clutch</u> + iem*it + <u>iem*clutch</u> + <u>iem*it*clutch</u> or sex + <u>clutch</u> + <u>iem</u> + sex*clutch + iem*sex + <u>iem*clutch</u> + <u>sex*clutch*iem</u> </div>	278	$F_{(250, 294)} = 1.8598$ $F_{(155, 554)} = 1.9261$	0.0001 0.0001
All juveniles at 10 months	it + clutch + iem + it*clutch + iem*it + iem*clutch + iem*it*clutch	278	$F_{(250, 299)} = 1.2887$	0.0179
Females only at hatching	it + clutch + <u>iem</u> + it*clutch + iem*it + iem*clutch + iem*it*clutch	170	$F_{(105, 132)} = 1.3758$	0.0414
Females only at 10 months	it + clutch + iem + <u>it*clutch</u> + iem*it + iem*clutch + <u>iem*it*clutch</u>	170	$F_{(105, 132)} = 1.6559$	0.0031
21°C females + 22°C males at hatching ¹	sex + <u>clutch</u> + <u>iem</u> + <u>sex*clutch</u> + iem*sex + <u>iem*clutch</u> + <u>iem*sex*clutch</u>	176	$F_{(110, 162)} = 1.9562$	0.0001
21°C females + 22°C males at 10 months ¹	sex + clutch + iem + sex*clutch + iem*sex + iem*clutch + iem*sex*clutch	176	$F_{(110, 166)} = 1.4540$	0.0145

¹ Where sex is listed, it could equally read incubation temperature, as results are identical. ² Underlining represents significance ($P < 0.05$) of hypothesis test results.

Table 3 cont.

(b) Models that best represent each size trait respectively from GLM procedure results ranked by AIC_c:

Data set	Best approximating models	n	K	AIC _c
<i>All juveniles at hatching</i>				
Jaw length	<u>it</u> + <u>clutch</u> + <u>iem</u> + <u>it*iem</u> or	277	55	-1572.7
	<u>sex</u> + <u>clutch</u> + <u>iem</u> + <u>sex*iem</u> or		53	-1571.3
	<u>it</u> + <u>clutch</u> + <u>iem</u>		53	-1573.7
Jaw width	<u>clutch</u> + <u>iem</u>		51	-1830.3
Snout-vent length	<u>it</u> + <u>clutch</u> + <u>iem</u> + <u>it*iem</u> or	277	55	212.6
	<u>it</u> + <u>clutch</u> + <u>iem</u>		53	210.6
Total length	<u>it</u> + <u>clutch</u> + <u>iem</u>		53	575.5
Weight	<u>it</u> + <u>clutch</u> + <u>iem</u> + <u>it*iem</u> or		55	-734.8
	<u>sex</u> + <u>clutch</u> + <u>iem</u> + <u>sex*iem</u>		53	-737.5
<i>All juveniles at 10 months</i>				
Jaw length	<u>it</u> + <u>iem</u> + <u>it*iem</u>	277	7	-1383.2
Jaw width	<u>it</u> + <u>iem</u> + <u>it*iem</u>		7	-1515.3
Snout-vent length	<u>it</u> + <u>iem</u> + <u>it*iem</u>		7	705.5
Total length	<u>it</u> + <u>iem</u> + <u>it*iem</u>		7	1474.2
Mass	<u>it</u> + <u>iem</u> + <u>it*iem</u>		7	470.2
<i>Females only at hatching</i>				
Jaw length	<u>it</u> + <u>iem</u> + <u>it*iem</u> or		5	-957.5
	<u>it</u> + <u>iem</u>		4	-959.7
Jaw width	<u>it</u> + <u>clutch</u> + <u>iem</u>		51	-1256.0
Snout-vent length	<u>it</u> + <u>clutch</u> + <u>iem</u> + <u>it*iem</u> or	170	52	162.0
	<u>it</u> + <u>iem</u> + <u>it*iem</u> or		5	161.7
	<u>it</u> + <u>clutch</u> + <u>iem</u> or		51	160.6
	<u>it</u> + <u>iem</u>		4	160.1
Total length	<u>it</u> + <u>clutch</u> + <u>iem</u> + <u>it*iem</u> or		52	331.9
	<u>it</u> + <u>clutch</u> + <u>iem</u>		51	332.1
Mass	<u>it</u> + <u>clutch</u> + <u>iem</u> + <u>it*iem</u> or		52	-528.2
	<u>it</u> + <u>clutch</u> + <u>iem</u> or		51	-529.8
	<u>clutch</u> + <u>iem</u>		50	-529.4

Table 3 (b) cont. Models that best represent each size trait respectively from GLM procedure results ranked by AIC_c:

<i>Females only at 10 months</i>				
Jaw length	<u>it</u> + <u>clutch</u> + iem + <u>it*iem</u>	52		-910.6
Jaw width	<u>it</u> + <u>iem</u> + it*iem or	5		-931.3
	<u>it</u> + <u>iem</u>	4		-932.4
Snout-vent length	<u>it</u> + <u>iem</u> + it*iem or	5		428.2
	<u>it</u> + <u>iem</u>	4	170	429.5
Total length	it + <u>iem</u> + it*iem or	5		885.0
	<u>it</u> + <u>iem</u>	4		882.8
Mass	<u>it</u> + <u>iem</u> + it*iem or	5		276.7
	<u>it</u> + <u>iem</u>	4		275.4
<i>21°C females + 22°C males at hatching</i>				
Jaw length	sex + <u>iem</u> + sex*iem or	5		-957.5
	sex + <u>iem</u>	4		-956.5
Jaw width	sex + <u>iem</u> + <u>sex*iem</u> or	5		-1104.4
	sex + <u>iem</u> or	4		-1102.6
	<u>iem</u>	3		-1101.9
Snout-vent length	sex + <u>iem</u> + sex*iem or	5	176	176.4
	sex + <u>clutch</u> + <u>iem</u> or	49		177.7
	sex + <u>iem</u> or	4		175.0
	<u>iem</u>	3		176.8
Total length	<u>clutch</u> + <u>iem</u>	48		423.7
Mass	sex + <u>iem</u> + <u>sex*iem</u>	50		-393.7
<i>21°C females + 22°C males at 10 months</i>				
Jaw length	sex + <u>iem</u> + sex*iem or	5		-883.9
	<u>sex</u> + <u>iem</u>	4		-883.1
Jaw width	sex + <u>iem</u> + sex*iem or	5		-962.3
	<u>sex</u> + <u>iem</u>	4		-961.8
Snout-vent length	sex + <u>iem</u> + sex*iem or	5	176	437.4
	<u>sex</u> + <u>iem</u>	4		437.1
Total length	<u>sex</u> + <u>iem</u> + <u>sex*iem</u>	5		946.5
Mass	sex + <u>iem</u> + sex*iem or	5		323.6
	sex + <u>iem</u> or	4		323.4
	<u>iem</u>	3		322.1

Figure 3. Jaw length of tuatara to ten months post-hatching by incubation treatment (constant artificial temperatures 18, 21 or 22°C; variable natural temperatures (Nat)).

Figure 4. Total length of tuatara to ten months post-hatching by incubation treatment (constant artificial temperatures 18, 21 or 22°C; variable natural temperatures (Nat)).

Figure 5. Snout-vent length of tuatara to ten months post-hatching by incubation treatment (constant artificial temperatures 18, 21 or 22°C; variable natural temperatures (Nat)).

Figure 6. Mass of tuatara to ten months post-hatching by incubation treatment (constant artificial temperatures 18, 21 or 22°C; variable natural temperatures (Nat)).

Figure 7. Jaw width of tuatara to ten months post-hatching by incubation treatment (constant artificial temperatures 18, 21 or 22°C; variable natural temperatures (Nat)).

Figure 8. SVL of artificially incubated juveniles at ten months was influenced by initial egg mass, incubation temperature (18, 21 or 22 °C) and the interaction of incubation temperature and initial egg mass.

Parental factors were the most common influences using the GLM/AIC_c procedure on all size traits of artificially incubated females at hatching (data set including all females from 18 and 21°C incubation treatments), except jaw length (Table 3 (b)). Incubation temperature was only a significant influence on jaw length and total length of hatchling females; mean size of these measurements was larger in 21°C females (Table 1).

Alternative models could explain other measurements without the significant influence of incubation temperature. By ten months of age, the influence of clutch identity was no longer apparent, except for jaw length. The primary influences on all measures of size of female juveniles at ten months were initial egg mass and incubation temperature; smaller eggs resulted in smaller juvenile females and warmer incubation temperatures produced larger females (Figure 8).

Initial egg mass was the primary influence on the data set consisting of hatchling females incubated at 21°C and hatchling males incubated at 22°C. Incubation temperature/sex was not a significant influence on any measure of size at hatching, but participated in models through interactions with initial egg mass to influence jaw width and mass. By ten months of age, incubation temperature/sex became a significant influence on all size traits except mass. Females from 21°C were on average larger than males incubated at 22°C (Table 1; Figures 3-6). These results could be interpreted in two ways: either sexual dimorphism is already subtly apparent, or 1°C is sufficient to influence the size of these traits by ten months of age. The interpretation of early detection of sexual dimorphism is unlikely, as the pattern is opposite to what would be expected, that is, adult females are smaller in length and head size than adult males, not larger (Dawbin 1982).

4.4.5. *Effects of Natural Incubation on Tuatara Size*

Size of naturally incubated hatchlings analysed using the MANOVA procedure was affected by the duration of incubation or mean temperature variable, in addition to sex, and interactions between the temperature variable and sex (Table 4 (a)). However, sex was only influential through the interaction terms. At ten months of age, additional factors that could equally explain the data included incubation time and clutch identity, and their interaction. Incubation time was only an influence through the interaction term with clutch. Sex was a significant factor in the model including sex, incubation time and their interaction. That is, females that incubated for longer were smaller at ten months (Figure 9). This pattern was not apparent for males. Note that all hatchlings were kept in similar conditions post-hatching.

Models that best described each size trait (respectively) of naturally incubated hatchlings using the GLM/AIC_c procedure all included a representative of the nest temperature variability (Table 4(b)). Constant temperature equivalent (cte) during February was included in models for size of jaw length and snout-vent length, while nest temperature variance was included in models of jaw width and mass. However, constant temperature equivalent in February and nest temperature variance are the temperature variables that best predicted sex (Chapter 3), indicating temperature factors and sex were confounded as for artificial incubation treatments. Hatchling SVL was maximised in natural nests with the cte between 21 and 23°C (Figure 10). Sex influenced jaw length, SVL and mass through interaction terms. Incubation time and clutch identity influenced total length and SVL. Females hatched from cooler nests, and embryos in cooler nests took longer to develop.

Table 4. Incubation temperature and parental effects on size of naturally incubated tuatara. Influences on size investigated include clutch, sex, and variables chosen to represent natural nest incubation temperatures: mean or variance throughout incubation (meant, vart), duration of incubation (inctime), or mean constant temperature equivalent in February (cte; calculated in Chapter 3).

(a) Models that best represent size data including jaw length, jaw width, SVL, total length (mm) and mass (g) using the MANOVA procedure:

Data set	Best approximating models	n	F approximation of Wilks' lambda statistic	P
Hatchlings	<u>inctime</u> + sex + <u>inctime*sex</u> or <u>meant</u> + sex + <u>meant*sex</u>	125	$F_{(5, 117)} = 4.3301$ $F_{(35, 427)} = 1.6205$	0.0012 0.0160
Juveniles at 10 months	<u>inctime</u> + sex + <u>inctime*sex</u> or <u>meant</u> + sex + <u>meant*sex</u> or <u>inctime</u> + <u>clutch</u> + <u>inctime*clutch</u>	124	$F_{(5, 116)} = 3.4664$ $F_{(35, 423)} = 1.5327$ $F_{(65, 410)} = 1.3394$	0.0059 0.0293 0.0499

(b) Models that best represent each size trait respectively from GLM procedure results ranked by AIC_c:

Data set	Best approximating models	n	K	AIC _c
<i>Hatchlings</i>				
Jaw length	<u>cte</u> + sex + <u>cte*sex</u>	125	26	-735.2
Jaw width	<u>vart</u>		18	-789.8
Snout-vent length	<u>cte</u> + sex + <u>cte*sex</u> or <u>inctime</u> + <u>clutch</u>		26 22	151.6 152.6
Total length	<u>inctime</u> + <u>clutch</u>		22	351.7
Mass	<u>vart</u> + sex + <u>sex*vart</u>		26	-159.5
<i>Juveniles at 10 months</i>				
Jaw length	<u>vart</u> or <u>vart</u> + sex + <u>sex*vart</u>	124	18 26	-578.8 -578.7
Jaw width	<u>meant</u>		13	-614.8
Snout-vent length	<u>vart</u>		18	373.0
Total length	<u>cte</u>		19	720.0
Mass	<u>vart</u>		18	209.9

Figure 9. Incubation period for naturally incubated juveniles. Females that incubated longer were smaller.

Figure 10. The influence of incubation temperature on size of naturally incubated hatchlings. Hatchling SVL was maximised in natural nests with the cte between 21 and 23°C.

At ten months of age, all measurements of juvenile size were influenced by some measure of incubation temperature. Nest temperature variance influenced jaw length, snout vent length and mass. Mean temperature of nests influenced jaw width. Total length at ten months was not a useful measurement for investigating influences on size, as all naturally incubated juveniles had lost part of their tail by this time (also affecting mass at ten months), compared to fewer incidences of tail-loss (~73%) and smaller amounts of tissue lost from tails of artificially incubated juveniles (Figure 6). An alternative model for jaw length included sex, but this factor was only influential through its interaction with nest temperature variance (Figure 11). Females from nests with greater temperature variance had smaller jaw lengths by ten months, than those from nests with smaller temperature variance. Males hatched from nests with greater variance in temperature, but contrary to expectations of effects on females, increased temperature variance did not result in smaller (or larger) jaw lengths by ten months.

Figure 11. The interaction between nest temperature variance and sex was a significant influence on jaw length. Females from more variable nests had smaller jaws.

4.4.6. Size of Tuatara: Natural Versus Artificial Incubation

Hatchlings from artificial and natural incubation environments had similar length (SVL and total length) and mass (Table 1, Figures 6 - 8). However, naturally incubated hatchlings had smaller heads (measured by jaw length and width; Figures 3 and 7) than artificially incubated hatchlings. By ten months of age, naturally incubated juveniles were significantly smaller in all measurements than those from the constant artificial treatments. Tail loss substantially reduced total length and probably mass in naturally incubated juveniles (Figure 6). Juveniles incubated at 21°C were larger than any other treatment group in all measurements after six months of age. Juveniles incubated at 18°C were the smallest of all artificially incubated young by ten months.

4.5. Discussion

4.5.1. Influences on Size of Tuatara

Parental

Parental effects predominantly influence size of hatchling tuatara, and they are still important at ten months of age. Initial egg mass is the most important factor affecting size of hatchlings from artificial incubation regimes. The influence of clutch identity may even have been underestimated as eggs in artificial treatments originated from 50 clutches (Chapter 2). Our method of ranking models penalised those including factors such as clutch, that had high degrees of freedom. Clutch size was small (mean = 7.6 eggs; Chapter 2), and the split-clutch design resulted in very small numbers of eggs from each clutch per treatment. Maternal influences on neonate size are consistent with all other groups of reptiles (Van Damme et al. 1992, Janzen 1993, Bobyn and Brooks 1994, Congdon et al. 1995, Reichling and Gutzke 1996). Parental factors also appear to

have important effects on hatchlings from natural nests but the experimental design limited their investigation. Initial egg mass was not recorded and clutch identity co-varied with incubation temperature variables as we wanted to disturb nests as little as possible to allow investigation of TSD in nature (Chapter 3).

Incubation temperature

Incubation temperature did not greatly influence size of hatchlings, although some differences are apparent depending on which measurement is being analysed, and by which method (MANOVA versus GLM/AIC_c). The influence of incubation temperature on hatchling size varies among groups of reptiles. Some studies demonstrate thermal influences on size (e.g., Phillips et al. 1990, Whitehead and Seymour 1990, Van Damme et al. 1992, Janzen 1993), while others do not (e.g., Packard and Phillips 1994, Reichling and Gutzke 1996, Booth 1998, Angilletta et al. 2000). Results vary even between studies of the same species (Brooks et al. 1991, Congdon et al. 1999), due to variation in experimental designs, types of analyses and factors analysed.

Incubation temperature significantly influences size by the time artificially incubated tuatara reach ten months of age, despite similar rearing conditions post-hatching.

Incubation temperature also affects growth of Cuban rock iguanas (*Cyclura nubila*) for the first 16 months post-hatching, despite little evidence of the influence of incubation temperature on size at hatching (Alberts et al. 1997). In tuatara, smaller eggs result in smaller hatchlings, and eggs incubated at 18°C produce smaller juveniles at ten months than similar sized eggs incubated at warmer temperatures. The 18°C treatment also compromises hatching success (Chapter 2). This treatment may be close to the lower

limit for successful incubation of tuatara eggs. Similar effects from suboptimal incubation regimes occur in crocodiles (Webb and Smith 1984), alligators (Joanen et al. 1987) and geckos (Tousignant and Crews 1995), although size differences are usually apparent at hatching in those species. Note, however, that juveniles incubated at 18°C are still larger by ten months of age than those incubated naturally.

The experimental design again limited investigation of the influence of temperature on size of hatchlings incubated naturally. Incubation temperature in natural nests was confounded with sex and parental effects, such as clutch identity. The sample size of eggs incubated naturally was considerably smaller than the number of eggs in artificial treatments, also lowering the power of analyses. Incubation temperatures in natural nests were measured at only one point, so may not represent variations in temperature experienced by eggs in distant parts of the nest. Water content of nests was not monitored.

Despite the experimental design, some interesting patterns emerged in sizes of naturally incubated juveniles. Hatchlings with the longest snout-vent lengths developed in nests with a constant temperature equivalent between 21 and 23°C. This corresponds well with the pivotal temperature for sex determination in constant artificial incubation conditions (between 21 and 22°C), and artificial incubation temperatures that produce the largest hatchlings by ten months. Males appear to be robust to thermal variation during embryonic development, that is, it has little effect on size. However, females that incubated for longer in natural nests were shorter on average than those that incubated for less time. Females from nests with greater variance in temperature had smaller jaw lengths than females from more stable nest temperatures, and than males in

nests with equivalent temperature variance. Thus, female embryos that develop in nests that have more stable thermal conditions, in more reliable sites for hatching, may be of higher quality. Different norms of reaction for each sex to thermal conditions are known in other species of reptiles (e.g., Shine et al. 1997). The female phenotype was more sensitive than the male phenotype to thermal regime in *Bassiana duperreyi*, a scincid species where sex is determined genetically (and hence not confounded with temperature; Elphick and Shine 1999). These patterns have been proposed as plausible explanations for the adaptive advantage of TSD (Charnov and Bull 1977, Shine et al. 1995).

Large jaw size has been linked to greater access to prey of a larger size range, giving those individuals a head start with respect to growth. Larger body size is supposedly advantageous for survival due to decreased vulnerability to gape-limited predators, and where food acquisition is competitive (Froese and Burghardt 1974, Fox 1978, McKnight and Gutzke 1993).

In species with nest-site philopatry, daughters should derive greater advantage from good nests, which they will eventually use themselves, than sons (Reinhold 1998). Female tuatara do aggressively compete for nest sites in discrete areas on Stephens Island: conflicts on rookeries can result in tail loss, other females often excavate eggs from nests during construction of their own nests, and females sometimes guard their nest sites, albeit for short periods of time relative to the nesting season (pers. obs. authors). However, nothing is known about the extent of nest-site philopatry in tuatara.

Although incubation duration does not affect size of hatchlings, incubation temperature may have an indirect effect on size of juveniles by controlling variation in hatching time in nature (e.g., Andrews et al. 2000); embryos at warmer temperatures hatch earlier in the season. The environment experienced by early hatchlings may be considerably different with respect to thermal conditions and availability of prey and refugia from that experienced by later hatchlings, and hence convey fitness advantages (Olsson et al. 1996, Sinervo and Doughty 1996). Juveniles that hatch earlier have a greater amount of time before winter to find refugia and grow. In tuatara, this pattern may be very important, as hatching occurs over at least five months (total incubation duration between 11-16 months (McIntyre 1997, pers. obs. NJN)); cooler tuatara nests may not hatch until the onset of winter, if at all (McIntyre 1997).

Resting metabolic rate of juvenile tuatara is significantly affected by ambient temperature (Cartland and Grimmond 1994). Temperature affects readiness to feed, gut passage time and digestive efficiency in cold-adapted New Zealand lizards (Lawrence 1997). In other species of reptiles, embryonic incubation temperatures influence juvenile temperature selection with consequences for growth (e.g., Huey 1982, Burger et al. 1987, O'Steen 1998). Growth rates of turtles diverged due to juvenile temperature selection, despite consumption of similar amounts of food and only 1°C difference in incubation temperature (O'Steen 1998). Hence a mechanism exists in nature for growth patterns witnessed in this study (under similar conditions) to be further exaggerated, where thermoregulatory opportunities exist and time of hatching has consequences for availability of resources.

4.5.2. Consequences of Size Differences for Tuatara

Consequences of incubation environment for quality of young can last months, even years in other reptiles (Joanen et al. 1987, Gutzke and Crews 1988, Burger 1989, Qualls and Shine 1996, Roosenburg and Kelley 1996, O'Steen 1998). However, quality is subjective. Assessing offspring quality by measuring body size alone may be misleading (Ford and Seigel 1994). Survival was not related to size in snapping turtles (*Chelydra serpentina*; Congdon et al. 1999). Furthermore, performance measures on snapping turtles were not necessarily intuitive. The less active hatchlings were more likely to survive their first year, as their cryptic colouration protected them from visual predators (Janzen 1995). Juvenile tuatara have cryptic colouration, but only limited work has been done on size-based performance; smaller tuatara tend to be faster (Pepperell 1982). Smaller lizards (*Sceloporus undulatus*, *Uta stansburiana*) are more likely to be non-survivors as they are more active in the wild; their territories have fewer refugia and/or less food, resulting in more exposure to predators (Ferguson et al. 1982).

4.5.3. Artificial Versus Natural Incubation

Artificial incubation conditions result in larger juveniles by ten months of age than natural incubation conditions, despite similar sizes at hatching, similar rearing conditions, and induction of half of the artificially incubated eggs (known to result in smaller egg size and hence smaller hatchlings; Chapter 2). Jaw sizes of young incubated in artificial conditions were larger at hatching than those from natural incubation conditions, potentially allowing greater access to food. However, naturally incubated young appeared to be more aggressive, with extensive tail loss caused by attacks from enclosure mates apparent from four months of age (pers. obs. NJN).

Naturally incubated tuatara were also housed in mixed sex ratios as a result of hatching dates, compared to one sex only in enclosures of artificially incubated tuatara. Effects of tail loss and enclosure sex ratio were not investigated here but could potentially result in the size patterns observed.

As size and performance data are not widely applicable amongst reptiles, and there is limited information for tuatara, we can only speculate on the longer term consequences of size differences. For example, alterations to size could affect reproductive capacity with consequences for lifetime reproductive success, especially as tuatara females have low annual reproductive output (approximately 2 eggs/female/year; Cree 1994). If age of first reproduction is size dependent, larger individuals may mature earlier. If maturity is age dependent, larger body size at maturity may result in the production of larger clutch sizes (Newman et al. 1994, Tyrrell et al. 2000). Either strategy could result in greater lifetime reproductive success (Roosenburg and Kelley 1996). It is not known whether female tuatara mature at a certain age or size.

Success of captive breeding and release programmes is dependent on the number and quality of offspring released into the wild (Ford and Seigel 1994). Performance experiments to test the significance of size in juvenile tuatara are needed (Chapter 5). If size of tuatara is a measure of quality, then those young produced by artificial incubation (including those from induced oviposition) are of better quality than those produced in more variable conditions in nature. However, the primary incubation temperature used for producing females to augment rare wild populations and provide founders for new populations of tuatara is the possibly marginal temperature of 18°C.

Females produced at this temperature are significantly smaller than those produced at 21°C, and suffer greater mortality during incubation (Chapter 2). Future artificial incubation to produce females may be better conducted at warmer temperatures, for example 20°C.

4.5.4. Sexual Dimorphism

A change of only 1°C during embryonic development alters the sex of hatchlings. This transition, although extremely subtle, is not unknown in other reptiles. For example, changes of 1-2°C alter the sex ratio of sea turtles (Mrosovsky and Yntema 1980).

Male and female gonads are distinguishable at hatching by internal examination or histology (Chapter 3). It is not possible to detect early divergence in sex dimorphism externally by overall size of tuatara at ten months of age. However, jaw size differences indicate support does exist for early divergence in characteristics that may later be useful in identifying sex; jaw size is smaller in females, on average, than in males.

4.5.5. Summary

Size of tuatara at hatching is primarily affected by egg mass, but incubation temperature influences size by ten months of age. Constant artificial incubation conditions result in larger juveniles by ten months of age, but they appear to be less aggressive than those from more variable natural incubation conditions. It is not known whether the “bigger is better” hypothesis applies to juvenile tuatara, but if so, artificial incubation is a good method for producing founders for new populations. However, a slight increase in the

primary female producing temperature may be prudent. Sexual dimorphism is not apparent at ten months of age, although some traits show signs of diverging, for example jaw size. Evidence from size patterns of tuatara incubated in natural nests support differential fitness models for the adaptive significance of TSD.

4.6. References

- Alberts, A. C., Perry, A. M., Lemm, J. M. and J. A. Phillips. 1997. Effects of incubation temperature and water potential on growth and thermoregulatory behaviour of hatchling Cuban rock iguanas (*Cyclura nubila*). *Copeia* 1997:766-776.
- Andrews, R. M., Mathies, T. and D. A. Warner. 2000. Effect of incubation temperature on morphology, growth and survival of juvenile *Sceloporus undulatus*. *Herpetological Monographs* 14:420-431.
- Angilletta, M. J. Jr., Winters, R. S. and A. E. Dunham. 2000. Thermal effects on the energetics of lizard embryos: implications for hatchling phenotypes. *Ecology* 81:2957-2968.
- Benton, M. J. 1990. Vertebrate Palaeontology: Biology and Evolution. HarperCollins Academic, London.
- Berry, J. F. and R. Shine. 1980. Sexual size dimorphism and sexual selection in turtles (Order Testudines). *Oecologia* 44:185-191.
- Bobyn, M. L. and R. J. Brooks. 1994. Interclutch and interpopulation variation in the effects of incubation conditions on sex, survival and growth of hatchling snapping turtles (*Chelydra serpentina*). *Journal of Zoology, London* 233:233-257.
- Booth, D. T. 1998. Incubation of turtle eggs at different temperatures: do embryos compensate for temperature during development? *Physiological Zoology* 71:23-26.
- Brooks, R. J., Bobyn, M. L., Galbraith, D. A., Layfield, J. A. and E. G. Nancekivell. 1991. Maternal and environmental influences on growth and survival of embryonic and hatchling snapping turtles (*Chelydra serpentina*). *Canadian Journal of Zoology* 69:2667-2676.
- Bull, J. J. 1980. Sex determination in reptiles. *The Quarterly Review of Biology* 55:4-21.
- Burger, J. 1989. Incubation temperature has long-term effects on behaviour of young pine snakes (*Pituophis melanoleucus*). *Behavioural and Ecological Sociobiology* 24:201-207.
- Burger, J. 1990. Effects of incubation temperature on behaviour of young black racers (*Coluber constrictor*) and kingsnakes (*Lampropeltis getulus*). *Journal of Herpetology* 24:158-163.
- Burger, J. and R. T. Zappalorti. 1988. Effects of incubation temperature on sex-ratios in pine snakes: differential vulnerability of males and females. *The American Naturalist* 132:492-505.

- Burger, J., Zappalorti, R. T., and M. Gochfeld. 1987. Effects of incubation temperature on hatchling pine snakes *Pituophis melanoleucus*. *Comparative Biochemistry and Physiology* 87A:727-732.
- Burnham, K. P. and D. R. Anderson. 1998. Model Selection and Inference. A Practical Information-theoretic Approach. Springer-Verlag NY Inc. USA.
- Cagle, K. D., Packard, G. C., Miller, K. and M. J. Packard. 1993. Effects of the microclimate in natural nests on development of embryonic painted turtles, *Chrysemys picta*. *Functional Ecology* 7:653-660.
- Cartland, L. K. and N. M. Grimmond. 1994. The effect of temperature on the metabolism of juvenile tuatara, *Sphenodon punctatus*. *New Zealand Journal of Zoology* 21:373-378.
- Charnov, E. L. and J. J. Bull. 1977. When is sex environmentally determined? *Nature* 266:828-830.
- Congdon, J. D., Fischer, R. U. and R. E. Gatten Jr. 1995. Effects of incubation temperatures on characteristics of hatchling American alligators. *Herpetologica* 51:497-504.
- Congdon, J. D., Nagle, R. D., Dunham, A. E., Beck, C. W., Kiney, O. M. and S. R. Yeomans. 1999. The relationship of body size to survivorship of hatchling snapping turtles (*Chelydra serpentina*): an evaluation of the "bigger is better" hypothesis. *Oecologia* 121:224-235.
- Cree, A. 1994. Low annual reproductive output in female reptiles from New Zealand. *New Zealand Journal of Zoology* 21:351-372.
- Cree, A. and D. Butler. 1993. Tuatara Recovery Plan (*Sphenodon* spp.). Threatened Species Recovery Plan Series No. 9. Department of Conservation, Wellington, New Zealand.
- Cree, A., Cockrem, J. F., Brown, M. A., Watson, P. R., Guillette, L. J. Jr., Newman, D. G. and G. K. Chambers. 1991. Laparoscopy, radiography, and blood samples as techniques for identifying the reproductive condition of female tuatara. *Herpetologica* 47:238-249.
- Cree A., Thompson, M. B. and C. H. Daugherty. 1995. Tuatara sex determination. *Nature* 375:543.
- Daugherty, C. H. 1998. From dark days to a bright future: survival of the tuataras. *ZooNooz* April:8-13.
- Dawbin, W. H. 1982. The tuatara *Sphenodon punctatus*: aspects of life history, growth and longevity. Pp. 237-250. In Newman, D. G. (Ed.). New Zealand Herpetology. New Zealand Wildlife Service Occasional Publication No. 2.

- Deeming, D. C. and M. W. J. Ferguson. 1991. Physiological effects of incubation temperature on embryonic development in reptiles and birds. Pp. 147-171. In Deeming, D. C. and M. W. J. Ferguson (Eds.). *Egg Incubation: its Effects on Embryonic Development in Birds and Reptiles*. Cambridge University Press, Cambridge.
- Dendy, A. 1899. Outlines of the development of the tuatara, *Sphenodon (Hatteria) punctatus*. *Quarterly Journal of the Microscopical Society* 42:1-87.
- Elphick, M. J. and R. Shine. 1998. Longterm effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards (*Bassiana duperreyi*, Scincidae). *Biological Journal of the Linnean Society* 63:429-447.
- Elphick, M. J. and R. Shine. 1999. Sex differences in optimal incubation temperature in a scincid lizard species. *Oecologia* 118:431-437.
- Ferguson, M. W. J. and T. Joanen. 1982. Temperature of egg incubation determines sex in *Alligator mississippiensis*. *Nature* 296:850-853.
- Ferguson, G. W. and S. F. Fox. 1984. Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. *Evolution* 38:342-349.
- Ferguson, G. W., Brown, K. L. and V. G. DeMarco. 1982. Selective basis for the evolution of variable egg and hatchling size in some iguanid lizards. *Herpetologica* 38:178-188.
- Ford, N. B. and R. A. Seigel. 1994. Phenotypic plasticity: implications for captive breeding and conservation programs. Pp 175-182. In Murphy, J. B., Adler, K. and J. T. Collins (Eds.). *Captive Management and Conservation of Amphibians and Reptiles*. Society for the Study of Amphibians and Reptiles, Ithaca (New York). Contributions to Herpetology. Volume 11.
- Fox, S. F. 1978. Natural selection on behavioural phenotypes of the lizard *Uta stansburiana*. *Ecology* 59:834-847.
- Froese, A. D. and G. M. Burghardt. 1974. Food competition in captive juvenile snapping turtles, *Chelydra serpentina*. *Animal Behaviour* 22:735-740.
- Gutzke, W. H. N. and D. Crews. 1988. Embryonic temperature determines adult sexuality in a reptile. *Nature* 332:832-834.
- Gutzke, W. H. N. and G. C. Packard. 1987. Influence of hydric and thermal environments on eggs and hatchlings of bull snakes *Pituophis melanoleucus*. *Physiological Zoology* 60:9-17.
- Head, G., May, R. M. and L. Pendleton. 1987. Environmental determination of sex in the reptiles. *Nature* 329:198-199.

-
- Huey, R. B. 1982. Temperature, physiology and the ecology of reptiles. Pp. 25-91. In Gans, C. and F. H. Pough (Eds.). *Biology of the Reptilia*. Academic Press, London.
- Janzen, F. J. 1993. The influences of incubation temperature and family on eggs, embryos, and hatchlings of the smooth softshell turtle (*Apalone mutica*). *Physiological Zoology* 66:349-373.
- Janzen, F. J. 1995. Experimental evidence for the evolutionary significance of temperature-dependent sex determination. *Evolution* 49:864-873.
- Janzen, F. J. 1996. Is temperature-dependent sex determination in reptiles adaptive? *Trends in Ecology and Evolution* 11:253.
- Joanen, T., McNease, L. and M. W. J. Ferguson. 1987. The effects of egg incubation temperature on post-hatching growth of American alligators. Pp. 533-537. In Webb, G. J. W., Manolis, S. C. and P. J. Whitehead (Eds.). *Wildlife Management: Crocodiles and Alligators*. Surrey Beatty, Sydney.
- Lang, J. W., Andrews, H. and R. Whitaker. 1989. Sex determination and sex ratios in *Crocodylus palustris*. *American Zoologist* 29:935-952.
- Lawrence, M. 1997. The effect of temperature on the eating behaviour, gut passage time and digestion efficiency of lizards. *New Zealand Journal of Zoology* 24:328 (abstract).
- McIntyre, M. 1997. *Conservation of the Tuatara*. Victoria University Press, Wellington.
- McKnight, C. M. and W. H. N. Gutzke. 1993. Effects of the embryonic environment and of hatchling housing conditions on growth of young snapping turtles (*Chelydra serpentina*). *Copeia* 1993:475-482.
- Miller, K., Packard, G. C. and M. J. Packard. 1987. Hydric conditions during incubation influence locomotor performance of hatchling snapping turtles. *Journal of Experimental Biology* 127:401-412.
- Mrosovsky, N. and C. L. Yntema. 1980. Temperature dependence of sexual differentiation in sea turtles: implications for conservation practices. *Biological Conservation* 18:271-280.
- Nelson, N. J. 1998. *Conservation of Brothers Island Tuatara, Sphenodon guntheri*. Unpublished MConSc Thesis, Victoria University of Wellington, New Zealand.
- Newman, D. G., Watson, P. R. and I. McFadden. 1994. Egg production by tuatara on Lady Alice and Stephens Islands. *New Zealand Journal of Zoology* 21:387-398.
- Olsson, M., Gullberg, A., Shine, R., Madsen, T. and H. Tegelstrom. 1996. Paternal genotype influences incubation period, offspring size, and offspring shape in an oviparous reptile. *Evolution* 50:1328-1333.

- O'Steen, S. 1998. Embryonic temperature influences juvenile temperature choice and growth rate in snapping turtles *Chelydra serpentina*. *Journal of Experimental Biology* 201:439-449.
- Packard, G. C. and M. J. Packard. 1988. The physiological ecology of reptilian eggs and embryos. Pp. 523-603. In Gans, C. and R. B. Huey (Eds.). *Biology of the Reptilia*. Volume 16. Ecology B.
- Packard, G. C. and J. A. Phillips. 1994. The importance of the physical environment for the incubation of reptilian eggs. Pp. 195-208. In Murphy, J. B., Adler, K. and J. T. Collins (Eds.). *Captive Management and Conservation of Amphibians and Reptiles*. Society for the Study of Amphibians and Reptiles, Ithaca (New York). *Contributions to Herpetology*. Volume 11.
- Pepperell, J. G. 1982. Tuatara *Sphenodon punctatus* locomotion: a summary. Pp. 207-211. In Newman, D. G. (Ed.). *New Zealand Herpetology*. New Zealand Wildlife Service Occasional Publication No. 2.
- Phillips, J. A., Garel, A., Packard, G. C. and M. J. Packard. 1990. Influence of moisture and temperature on eggs and embryos of green iguanas (*Iguana iguana*). *Herpetologica* 46:238-245.
- Qualls, C. P. and R. Shine. 1996. Reconstructing ancestral reaction norms: an example using the evolution of reptilian viviparity. *Functional Ecology* 10:688-697.
- Reichling, S. B. and W. H. N. Gutzke. 1996. Phenotypic consequences of incubation environment in the African Elapid genus *Aspidelaps*. *Zoo Biology* 15:301-308.
- Reinhold, K. 1998. Nest-site philopatry and selection for environmental sex determination. *Ecological Evolution* 12:245-250.
- Rhen, T. and J. W. Lang. 1995. Phenotypic plasticity for growth in the common snapping turtle: effects of incubation temperature, clutch, and their interaction. *The American Naturalist* 146:726-747.
- Roosenburg, W. M. and K. C. Kelley. 1996. The effect of egg size and incubation temperature on growth in the turtle, *Malaclemys terrapin*. *Journal of Herpetology* 30:198-204.
- Schwarzkopf, L. and R. J. Brooks. 1987. Nest-site selection and offspring sex ratio in painted turtles, *Chrysemys picta*. *Copeia* 1987:53-61.
- Shine, R. 1983. Reptilian viviparity in cold climates: testing the assumptions of an evolutionary hypothesis. *Oecologia* 57:397-405.
- Shine, R. 1999. Why is sex determined by nest temperature in many reptiles? *Trends in Ecology and Evolution* 14:186-189.
- Shine, R., Elphick, M. J. and P. S. Harlow. 1995. Sisters like it hot. *Nature* 378:451-452.

- Shine, R., Elphick, M. J. and P. S. Harlow. 1997. The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology* 78:2559-2568.
- Shine, R. and P. S. Harlow. 1996. Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology* 77:1808-1817.
- Sinervo, B. and P. Doughty. 1996. Interactive effects of offspring size and timing of reproduction: experimental, maternal, and quantitative genetic aspects. *Evolution* 50:1314-1327.
- Spotila, J. R., Zimmerman, L. C., Binckley, C. A., Grumbles, J. S., Rostal, D. C., List, A. Jr., Beyer, E. C., Phillips, K. M. and S. J. Kemp. 1994. Effects of incubation conditions on sex determination, hatching success, and growth of hatchling desert tortoises, *Gopherus agassizii*. *Herpetological Monographs* 8:103-116.
- Steyermark, A. C. and J. R. Spotila. 2000. Effects of maternal identity and incubation temperature on snapping turtle (*Chelydra serpentina*) metabolism. *Physiological Zoology and Biochemistry* 73:298-306.
- Steyermark, A. C. and J. R. Spotila. 2001. Effects of maternal identity and incubation temperature on hatching and hatchling morphology in snapping turtles, *Chelydra serpentina*. *Copeia* 2001:129-135.
- Swingland, I. R. and M. J. Coe. 1979. The natural regulation of giant tortoise populations on Aldabra Atoll: recruitment. *Philosophical Transactions of the Royal Society, London Series B* 286:177-188.
- Thompson, M. B. 1990. Incubation of eggs of tuatara, *Sphenodon punctatus*. *Journal of Zoology, London* 222:303-318.
- Thompson, M. B., Newman, D. G. and P. R. Watson. 1991. Use of oxytocin in obtaining eggs from tuatara (*Sphenodon punctatus*). *Journal of Herpetology* 25:101-104.
- Thompson, M. B., Packard, G. C., Packard, M. J. and B. Rose. 1996. Analysis of the nest environment of tuatara *Sphenodon punctatus*. *Journal of Zoology, London* 238:239-251.
- Tousignant, A. and D. Crews. 1995. Incubation temperature and gonadal sex affect growth and physiology in the leopard gecko (*Eublepharis macularis*), a lizard with temperature-dependent sex determination. *Journal of Morphology* 224:159-170.
- Tyrrell, C. L., Cree, A. and D. R. Towns. 2000. Variation in reproduction and condition of northern tuatara (*Sphenodon punctatus punctatus*) in the presence and absence of kiore. Science for Conservation 153. Department of Conservation, New Zealand.

- Van Damme, R., Bauwens, D., Brana, F. and R. F. Verheyen. 1992. Incubation temperature differentially affects hatching time, egg survival and sprint speed in the lizard *Podarcis muralis*. *Herpetologica* 48:220-228.
- Vinegar, A. 1974. Evolutionary implications of temperature induced anomalies of development in snake embryos. *Herpetologica* 30:72-74.
- Webb, G. J. W., Beal, A. M., Manolis, S. C. and K. E. Dempsey. 1987. The effects of incubation temperatures on sex determination and embryonic development rate in *Crocodylus johnstoni* and *C. porosus*. Pp. 507-531. In Webb, G. J. W., Manolis, S. C. and P. J. Whitehead (Eds.). *Wildlife Management: Crocodiles and Alligators*. Surrey Beatty, Sydney.
- Webb, G. J. W. and A. M. A. Smith. 1984. Sex ratio and survivorship in the Australian freshwater crocodile *Crocodylus johnstoni*. *Symposium of the Zoological Society of London* 52:319-355.
- Whitehead, P. and R. S. Seymour. 1990. Patterns of metabolic rate in embryonic crocodilians *Crocodylus johnstoni* and *Crocodylus porosus*. *Physiological Zoology* 63:334-352.

Chapter 5. Performance and potentially fitness of tuatara depend on incubation regime and length of juveniles

Nicola J. Nelson¹, Michael B. Thompson², Susan N. Keall¹ and Charles H. Daugherty¹

¹ School of Biological Sciences, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand; ² School of Biological Sciences and Wildlife Research Institute, Heydon-Laurence Building (A08), University of Sydney, NSW 2006, Australia.

5.1. Abstract

Tuatara are sexually dimorphic reptiles with temperature-dependent sex determination. Artificial incubation is used to produce founders for new populations of tuatara as they are of high conservation importance. We investigate whether size and incubation treatment induce performance differences that might affect fitness of juvenile tuatara (*Sphenodon punctatus*). We examine performance measures that could be closely connected to survival and growth of tuatara in natural habitats: sprint speed, predator avoidance and willingness to feed. Performance is affected by incubation regime (natural versus artificial incubation). Naturally incubated juveniles are the smallest animals from any incubation regime, but at ten months of age these juveniles are the fastest runners. Naturally incubated juveniles are more likely to respond to predator stimuli by running, and at ten months of age are slightly more willing to feed and faster to feed under the test conditions than those incubated artificially. Some aspects of performance of juvenile tuatara change over time. Older juveniles run faster than younger juveniles. Older juveniles are more likely to respond to predator stimuli by running. Performance of juvenile tuatara can vary with size. Longer juveniles are faster than shorter juveniles of the same age, but they also demonstrate the greatest range in sprint speeds. We cannot determine whether performance is directly affected by incubation temperature, as incubation temperature co-varies with sex. Artificially

incubated juveniles do well in captivity. However, naturally incubated juvenile tuatara are more aggressive, faster for their size, and their reaction norm to predator stimuli is to run, suggesting they may be more fit in the wild than artificially incubated juveniles.

Keywords: Tuatara, *Sphenodon punctatus*, performance, fitness, juvenile size, incubation.

5.2. Introduction

Biological fitness has been defined as both the contribution an organism makes to the next generation and also its relative lifetime reproductive success. Phenotypic differences affect the relative fitness of individuals (Ridley 1997). Incubation temperatures during embryonic development can affect phenotypic traits such as size and performance of reptile hatchlings (e.g. Burger 1989, Burger 1990, Van Damme et al. 1992, Rhen and Lang 1995, Downs and Shine 1999, Elphick and Shine 1999). For phenotype at hatching to affect fitness, phenotypic differences must affect either probability of survival to reproductive age or success in reproduction, for example, size at maturity (Ferguson and Fox 1984, Stearns and Koella 1986, Van Damme et al. 1992, Rhen and Lang 1995).

Willingness to feed (Shine et al. 1997), running speed (Elphick and Shine 1998, Van Damme et al. 1992, Qualls and Andrews 1999), and predator avoidance tactics (Burger 1989) are examples of performance measures used to assess behavioural responses with respect to embryonic incubation regimes. Feeding performance and sprint speed also potentially affect growth and survival. Vulnerability to predation is a plausible

mechanism by which phenotypic differences could affect fitness, because predation is implicated as a major selective force in the evolution of morphological and behavioural characteristics of animals (Green 1988, Lima and Dill 1990, Downs and Shine 1999). However, responses of animals to these tests are not necessarily consistent among species. For example, running speed may vary with incubation temperature, depending on the study animal (e.g. Elphick and Shine 1998 versus Van Damme et al. 1992). Also, neither the precise magnitude of a response nor the direction of the response may be important in its own right, for example, bigger does not necessarily equal better (Chiszar et al. 1993, Congdon et al. 1999). Size and incubation effects may also disappear over time (Andrews et al. 2000).

Tuatara (*Sphenodon* spp.) are medium-sized, sexually dimorphic reptiles with temperature-dependent sex determination (TSD; Dawbin 1982, Cree et al. 1995). Now restricted to offshore islands of New Zealand, they are of high conservation importance (Cree and Butler 1993), and are biologically significant as the sole living representatives of the reptilian order Sphenodontia (*sensu* Benton 1990, Cree and Butler 1993). Eggs have been collected and artificially incubated to produce founders for new captive and wild populations, and to augment existing rare populations (Nelson 1998, Daugherty 1998). Incubation regime (natural versus artificial) and temperature during embryonic development do not affect size of tuatara (*S. punctatus*) at hatching. By ten months of age, however, juveniles from warmer artificial incubation treatments are larger (snout-vent length and mass) than those from cooler treatments and juveniles incubated in natural nests (Chapter 4). Juveniles incubated in natural nests have a greater incidence of tail loss than those from artificially incubated eggs.

This chapter investigates whether size and incubation treatment affect performance in ways that might affect fitness of juvenile tuatara. We ask the following questions:

1. Does performance vary with size?
2. Is performance related to incubation temperature?
3. Do juveniles from artificial incubation regimes show similar behavioural responses to those from natural incubation regimes?
4. Do performance patterns change by ten months post-hatching?

We examine performance measures that could be closely connected to survival and growth of tuatara in natural habitats: sprint speed, predator avoidance and willingness to feed. Tuatara are capable of short sprints but tend to move in a stop-start manner, avoiding predators by retreating quickly into burrows, and catching prey by sit-and-wait tactics (Daugherty and Cree 1990). For these reasons we assessed sprint speed over a short distance and make the assumption that faster animals will be more likely to survive and obtain food. The emphasis during predator avoidance tests is on relative qualitative responses among incubation groups of animals, rather than the magnitude of the responses. Running or freezing responses are both feasible tactics for predator avoidance. Tuatara that retreat quickly into burrows are able to avoid native aerial predators (Daugherty and Cree 1990). Tuatara that freeze may be difficult for predators to detect due to their cryptic colouration (Dawbin 1982). Typical terrestrial predators include adult tuatara which identify potential food items by movement (Dawbin 1982, Daugherty and Cree 1990). Responses are compared to the reaction norm for naturally incubated juveniles. For tests of willingness to feed, we assume that those juveniles most willing to feed would be the most successful in nature.

5.3. Methods

5.3.1. *Source Animals, Test Times and Conditions*

Artificial incubation was conducted at three temperatures: 18, 21, and 22°C (Chapter 2). Natural nests experienced variable temperatures throughout incubation (Chapter 3). See Chapters 2 and 3 for details of the study site, methods for collection of eggs, incubation conditions, post-hatching rearing conditions, and the method used to identify sex of juveniles. Performance of juvenile tuatara (*S. punctatus*) from controlled artificial incubation conditions and natural nests was measured at one, six and ten months post-hatching.

All juvenile tuatara were housed individually, without food, in the same room in which performance tests were conducted for three days prior to assessment of sprint speed and predator avoidance. Juveniles had no experience of natural predators. Before testing commenced, the following measurements were taken: snout-vent length (SVL; mm), total length (TL; mm), mass (g), jaw width (mm), and jaw length (mm). Performance testing was conducted at room temperature (which was recorded and used in analyses), and centred around midday. Although adult tuatara are mainly active at night, juveniles are also active during the day. Experimental group sizes varied because every juvenile was tested within three days of their one, six and ten month hatch dates, hence a universal start and finish time for experimentation was not feasible. Juveniles were held for a further three days, that is, a total of six days, at which time their willingness to feed was measured. The same person conducted all tests and measurements (NJN).

5.3.2. *Performance Measures*

Sprint speed

Sprint speed was measured on a 1.5 m straight, flat racetrack using an electronic stopwatch. A stopwatch was wired to five pairs of infrared transmitters and receivers placed 0.25 m apart along, and 4 mm above the floor of the track. When the tuatara crossed the first set of lights, the stopwatch began timing on four panels. Each timer stopped when its respective beam was crossed, providing four measurements for the time taken (in seconds) to run each 0.25 m stretch (or the total time taken to run 1 m). Approximately 0.2 m was available at one end of the track for placing the tuatara prior to testing, and 0.3 m at the other end of the track for the animal to run onto. Tuatara were encouraged to run along the racetrack using a paintbrush to flick their tails. Three tests were conducted on each animal, consecutively, without a rest period.

Predator avoidance

Predator avoidance was tested in a rectangular glass terrarium (300 mm by 600 mm). The sides of the terrarium were covered with black plastic. The substrate consisted of dry soil, and two small pieces of bark were provided as shelters. Each tuatara was placed at the same starting place, facing the same direction in the terrarium for three separate tests. Firstly, their reaction to handling was recorded as either a freezing or running response upon release into the terrarium. Secondly, once the animal had stopped running, its response to a ground predator was assessed. The ground predator consisted of a quick grab at the animal by the researcher using her right hand. Again, the response was recorded as “freeze” or “run”. The tuatara was then placed in the same position in the terrarium as it was initially released. Any that moved off the site were repositioned. The third test was of the juveniles’ response to an aerial predator. This

was measured by passing a two dimensional cardboard life-size cutout of a morepork (*Ninox novaeseelandiae*) on a stick over the terrarium. Moreporks are nocturnal aerial avian predators native to New Zealand that take prey the size of juvenile tuatara (e.g. lizards; Falla et al. 1978). Aerial predator tests were conducted three times in quick succession. “Freeze” or “run” responses were recorded. Any tuatara that moved off the test site were repositioned. The researcher always sat in the same position with respect to the terrarium, directly behind the initial test position. Predator avoidance tests were always conducted after all tuatara in the test group had undergone sprint tests, and individuals were tested in the same sequence as both measurements and sprint tests were conducted.

Willingness to feed

Up to five white 4 L buckets at a time, each containing four small black cricket nymphs, were placed beneath a video camera linked to a television screen and video recorder. A digital timer was placed beside the buckets. The video recorder was started, then one tuatara was released quickly by hand into each bucket, and the timer was started. The interval of time after placing the tuatara into the bucket until their first lunge for a cricket was recorded (up to ten minutes).

5.3.3. Statistical Analyses

Data from only the one and ten month performance tests were statistically analysed. Measurements taken when juveniles were six months old are included in some figures to demonstrate trends. Effects of induction of oviposition on performance were not specifically analysed. However, induced eggs produced smaller juveniles, and size was included in all analyses.

Sprint speed

Analyses of variance and covariance (ANOVA, ANCOVA) using the GLM procedure in SAS 6.12 were conducted on the fastest sprint speed (ms^{-1}) of each tuatara over 0.25 m. Analyses on all hatchlings at one and ten months included incubation treatment (i.e. artificial versus natural incubation regime) and sex as fixed categorical factors.

Covariates used were continuous measures of size (SVL and TL) and test temperature.

A subset of the data including only sprint speeds of artificially incubated juveniles at one and ten months was analysed using incubation temperature as a fixed categorical factor. We acknowledge pseudo-replication may be a problem with respect to analyses of incubation temperature, because each treatment was carried out in only one incubator. However, this is a standard technique used for incubation of reptile eggs.

Sex was not included in these analyses as incubation temperatures co-varied with sex of hatchlings (Chapters 2 and 4). Covariates used were continuous measures of size (SVL and TL) and test temperature. Similarly, a subset of the data including only speeds of naturally incubated juveniles at one and ten months was analysed. In this instance, however, incubation temperature was treated as a random effect. Constant temperature equivalent (cte; Chapter 3) in February was the variable used to represent the incubation temperature of each nest.

All data sets satisfied the assumptions of normality and homogeneity of variances. The few outliers that were identified were retained in the datasets.

Predator avoidance

Categorical predator avoidance data were analysed by performing logistic regressions using the probit procedure in SAS 6.12. The dependent variables were run or freeze

responses to handling, to the ground predator, and to the aerial predator, respectively. We analysed data sets including all animals, a subset of artificially incubated juveniles only, and a subset of naturally incubated juveniles only, at one and ten months of age. We included the same suite of factors and covariates used in sprint analyses. However, the probit procedure could not treat incubation temperature of natural nests as a random effect. To test whether incubation temperature in natural nests significantly affected responses to handling and predators, we used the 'nlmixed' procedure in SAS 8.2. We tested models with and without the random incubation temperature effect. Significance was determined by subtracting the residual deviance of the model including incubation temperature from the residual deviance of the model without incubation temperature. Test statistics greater than 1.92 were significant at the 5% level (Self and Liang 1987). We report on models only where convergence criteria were satisfied.

Willingness to feed

Whether a juvenile fed or not within the ten minute test period was analysed by performing logistic regressions using the probit procedure in SAS 6.12. We analysed the same groups of data and the same suite of factors and covariates used in the predator avoidance procedure. In addition, mass of juveniles was included as a covariate. Again, the significance of effects of natural incubation temperatures was analysed using the 'nlmixed' procedure in SAS 8.2.

If juveniles fed within the test period, then the time taken to lunge for the first time at an item of food was analysed using the GLM procedure in SAS 6.12. Analyses of the whole data set included incubation treatment (artificial versus natural) and sex as fixed factors. We included incubation temperature as a fixed factor in analyses of a subset of

artificially incubated juveniles. Incubation temperature of natural nests (using the constant temperature equivalent (cte) of each nest calculated in Chapter 3) was treated as a random effect in analyses including naturally incubated juveniles only. We included SVL, TL, and mass as covariates in all analyses. The feeding response of juveniles was analysed at one and ten months of age.

Maternal effects

Maternal effects were not directly included in analyses of performance. Initial egg mass was the most influential factor on size of hatchlings and was still an important determinant of size at ten months of age (Chapters 2 and 4). Clutch identity may also be important in determining size of juvenile tuatara (Chapter 4). Hence, size was used as a covariate in all analyses of performance.

5.4. Results

5.4.1. Sprint Speed

Sprint speeds of juveniles at one month of age ranged from 0.20 to 1.11 ms⁻¹ (Table 1) and 0.24 to 3.16 ms⁻¹ at ten months post-hatching (Table 2). Mean sprint speeds increased with age (Tables 1 and 2).

Factors that significantly influenced sprint speed of all juveniles at one month of age were snout-vent length ($F_{(1)} = 4.30$; $p = 0.0388$) and total length ($F_{(1)} = 7.44$; $p = 0.0067$; Figure 1). Longer juveniles ran faster and showed a greater range of speeds. In addition, incubation temperature affected sprint speed of naturally incubated juveniles at one month post-hatching ($F_{(17)} = 4.37$; $p = 0.0001$; Figure 2). The fastest and greatest

range of sprint speeds was recorded for juveniles incubated at intermediate temperatures (21-23 °C cte).

At ten months of age, sprint speed of all juveniles was significantly influenced by incubation treatment (Figure 3) and test temperature ($F_{(2)} = 18.10$; $p = 0.0001$).

Naturally incubated juveniles ran significantly faster than artificially incubated juveniles. Sprint speed of artificially incubated juveniles was weakly, but significantly affected by test temperature ($F_{(1)} = 10.27$; $p = 0.0015$; Figure 4). Warmer test temperatures resulted in a slight increase in sprint speeds. Sprint speed of naturally incubated juveniles was only weakly affected by snout-vent length ($F_{(1)} = 8.80$; $p = 0.0036$; Figure 5). Longer juveniles had a greater range of sprint speeds than shorter juveniles.

Table 1. Summary of the fastest sprint speeds (ms^{-1}) of juveniles at one month post-hatching over a distance of 0.25 m. Data categories are listed by incubation (inc.) regime.

Data Category	N	Min.	Max.	Mean	SE
Artificial inc.	278	0.22	1.11	0.49	0.01
Natural inc.	124	0.20	0.98	0.47	0.01
Male	186	0.20	1.02	0.48	0.01
Female	215	0.24	1.11	0.49	0.01
<i>Artificial inc.</i>					
18°C	99	0.30	1.11	0.51	0.02
21°C	74	0.35	0.91	0.49	0.02
22°C	105	0.22	1.02	0.48	0.01
<i>Natural inc.</i>					
Male	78	0.20	0.98	0.49	0.02
Female	46	0.24	0.77	0.44	0.02

Table 2. Summary of the fastest sprint speed (ms^{-1}) of juveniles at ten months post-hatching over a distance of 0.25 m.

Data Category	N *	Min.	Max.	Mean	SE
Artificial inc.	277	0.24	1.56	0.67	0.02
Natural inc.	123	0.25	3.16	0.84	0.03
Male	183	0.24	3.16	0.77	0.03
Female	216	0.25	2.46	0.69	0.02
<i>Artificial inc.</i>					
18°C	99	0.33	1.13	0.64	0.02
21°C	74	0.25	1.07	0.67	0.03
22°C	104	0.24	1.56	0.72	0.03
<i>Natural inc.</i>					
Male	76	0.25	3.16	0.85	0.05
Female	46	0.37	2.46	0.83	0.05

* Differences in the number of individuals between one (Table 1) and ten month tests were primarily due to equipment failure rather than deaths. If equipment failed, individuals were not re-tested.

Figure 1. Sprint speeds of juvenile tuatara at 1 month of age as a function of total length.

Figure 2. Sprint speeds of naturally incubated juveniles at one month of age as a function of incubation temperature.

Figure 3. Mean ($\pm$ SE) of fastest speeds of juvenile tuatara at one, six and ten months post-hatching over 0.25 m. Juveniles are grouped by artificial (18, 21 and 22°C) and natural incubation regime (var).

Figure 4. Sprint speed of artificially incubated juveniles at ten months of age as a function of test temperature.

Figure 5. Sprint speed of naturally incubated juveniles at ten months of age as a function of snout-vent length.

5.4.2. Predator Avoidance

Response to handling

At one month post-hatching, the response of most juveniles to handling was to freeze (Table 3). The only factor that significantly affected behaviour of juveniles to handling was incubation temperature in natural nests (*Test statistic* = 2.1; $p < 0.05$).

Table 3. Response of one month old juveniles to handling.

Behaviour	Data Sets:		
	<i>All juveniles</i>	<i>Artificially inc.</i>	<i>Naturally inc.</i>
Ran	14	7	7
Froze	387	270	117

At 10 months post-hatching, the response to handling was more variable (Table 4). For example, half of the naturally incubated juveniles froze while the other half ran off the placement site. Factors that affected response to handling included SVL and test temperature. Longer juveniles were more likely to freeze (Figure 6). Intermediate test temperatures were more likely to result in a running response to handling (Figure 7). Sex was only an influential factor when combined with snout-vent length, test temperature or incubation treatment (i.e. artificial versus natural incubation; Table 4).

The response of the subset of artificially incubated juveniles at ten months of age was affected by incubation temperature. Higher incubation temperatures resulted in juveniles that were more likely to freeze in response to handling (43% froze at 18°C, 61% at 21°C, and 78% at 22°C). Snout-vent length and test temperature also influenced behaviour when included in analyses with incubation temperature (Table 4).

The response of the subset of naturally incubated juveniles at ten months of age was affected either by test temperature or sex (Table 4), and incubation temperature (*Test statistic* = 4.6; $p < 0.05$). Increasing test temperature resulted in an increasing proportion of juveniles responding to handling by running. More males ran than females.

Table 4. Response of ten month old juveniles to handling.

Data Categories	N	Ran	Froze	Significant Factors	$X^2_{(df)}$	Pr>Chi
All juveniles	402	171	231	Snout-vent length	$X^2_{(1)} = 13.3$	0.0003
				Expt. temp. ¹	$X^2_{(1)} = 9.5$	0.0019
				Inc. tmt. ²	$X^2_{(1)} = 7.1$	0.0075
				+ sex	$X^2_{(1)} = 4.9$	0.0275
				Sex	$X^2_{(1)} = 6.5$	0.0107
				+ expt. temp.	$X^2_{(1)} = 13.1$	0.0003
				Sex	$X^2_{(1)} = 5.5$	0.0195
				+ expt. temp.	$X^2_{(1)} = 7.9$	0.0050
				+ SVL	$X^2_{(1)} = 8.0$	0.0046
Artificial inc. only	278	108	170	Inc. temp.	$X^2_{(2)} = 24.4$	0.0001
				Inc. temp.	$X^2_{(2)} = 16.6$	0.0003
				+ SVL	$X^2_{(1)} = 3.9$	0.0491
				Inc. temp.	$X^2_{(2)} = 27.8$	0.0001
				+ expt. temp.	$X^2_{(1)} = 7.4$	0.0067
				Expt. temp	$X^2_{(1)} = 4.4$	0.0368
				Sex	$X^2_{(1)} = 7.3$	0.0069

¹ Expt. temp. refers to the room temperature at which the test was carried out; ² Inc. tmt. refers to incubation treatment, that is, artificial versus natural incubation.

Figure 6. Response of ten month old juveniles to handling as a function of snout-vent length (1 = freeze; 2 = run). Note: Curves were included to illustrate where hidden data points exist on the graph.

Figure 7. Response of ten month old juveniles to handling as a function of test temperature (1 = freeze; 2 = run). Note: Curves were included to illustrate where hidden data points exist on the graph, notably at intermediate test temperatures where more juveniles responded to handling by running than freezing.

Response to a ground predator

At one month of age, 60% of all juveniles froze in response to a ground predator (Table 5). The only significant factor influencing behaviour was incubation treatment ($n = 401$; $X^2_{(1)} = 20.5572$; $Pr > Chi = 0.0001$). Naturally incubated juveniles were more likely to run than those from the artificial incubation treatment. Incubation temperature

significantly affected the response of naturally incubated juveniles (*Test statistic* = 3.0; $p < 0.05$).

By ten months post-hatching most juveniles responded to a ground predator by running (Table 5). Test temperature became an additional significant influence on behaviour of juveniles towards a ground predator ($n = 402$; $X^2_{(1)} = 10.712$; $Pr > Chi = 0.0011$).

Increased test temperature increased the likelihood of running. Incubation temperature was no longer a significant influence on behaviour of naturally incubated juveniles (*Test statistic* = 0.5; $p > 0.05$).

Table 5. Behaviour of juveniles in response to a ground predator.

Behaviour	Age (post-hatching)	Data Set: <i>All juveniles</i>	<i>Artificially inc.</i>	<i>Naturally inc.</i>
Ran	1 month	159	89	70
Froze	1 month	242	188	54
Ran	10 months	344	227	117
Froze	10 months	58	51	7

Response to an aerial predator

Fifty-four percent of juveniles at one month of age responded to an aerial predator by running (Table 6). A larger percentage of naturally incubated males ran than females ($n = 124$; $X^2_{(1)} = 13.8319$; $Pr > Chi = 0.0002$). Incubation temperature significantly influenced behaviour of naturally incubated juveniles (*Test statistic* = 8.9; $p < 0.05$). At ten months post-hatching, 66% of the juveniles ran in response to the aerial predator

(Table 6). The only significant factor influencing behaviour of all juveniles was incubation treatment ($n = 402$; $X^2_{(1)} = 16.0372$; $Pr > Chi = 0.0001$). Naturally incubated juveniles were more likely to run in response to an aerial predator than juveniles from artificial incubation treatments. Sex significantly influenced behaviour of naturally incubated juveniles ($n = 124$; $X^2_{(1)} = 7.6542$; $Pr > Chi = 0.0057$). A larger percentage of males ran than females.

Table 6. Behaviour of juvenile tuatara in response to an aerial predator.

Behaviour	Age (post-hatching)	Data Set: <i>All juveniles</i>	<i>Artificially inc.</i>	<i>Naturally inc.</i>
Ran	1 month	218	150	68
Froze	1 month	183	127	56
Ran	10 months	266	166	100
Froze	10 months	136	112	24

5.4.3. *Willingness to Feed*

Less than half of the artificially incubated juveniles fed within the ten minute test period at one and ten months post-hatching. Only 27% of the naturally incubated juveniles fed at one month of age, but 66% of the same animals responded at ten months (Table 7). Hence, incubation treatment affected whether juveniles fed or not at one and ten months of age. Sex was a significant factor affecting feeding only in conjunction with incubation treatment in the one month tests. Incubation temperature affected the response to food of artificially incubated juveniles at one month of age. However, sex and incubation temperature co-vary due to TSD. The 22°C treatment consisted only of

males, and these juveniles were less likely to feed than juveniles from female producing temperatures (18 and 21°C).

Table 7. Summary of feeding trials and factors that affected whether juveniles fed within the ten minute test period.

Data Categories	N	% Fed	Significant Factors	Chi Square	Pr>Chi
<i>1 month</i>					
All juveniles	403	41	Inc. tmt. ¹	$X^2_{(1)} = 13.322$	0.0003
			Sex	$X^2_{(1)} = 12.777$	0.0004
			Inc. tmt.	$X^2_{(1)} = 9.139$	0.0025
			+ sex	$X^2_{(1)} = 8.373$	0.0035
Artificial inc. only	278	47	Inc. temp. ²	$X^2_{(2)} = 11.985$	0.0025
Natural inc. only	125	27	-----		
<i>10 months</i>					
All juveniles	398	46	Inc. treatment ¹	$X^2_{(1)} = 28.197$	0.0001
Artificial inc. only	274	37	-----		
Natural inc. only	124	66	-----		

¹ Inc. tmt. refers to artificial versus natural incubation; ² Inc. temp. refers to artificial incubation temperature (i.e. 18, 21 and 22°C).

Of those juveniles that did feed, the time taken to attempt to secure a prey item ranged from 0 to 592 s (Tables 8 and 9) from an entire test period of 600 s. None of the factors investigated affected the feeding time of one month old juveniles. Factors that affected

feeding time at 10 months of age included incubation treatment and various measures of size (jaw length, SVL and mass; Table 10). Naturally incubated juveniles responded to food slightly faster than juveniles from the artificial incubation regime. The time taken to respond to food increased to some extent with increasing snout-vent length (e.g. Figure 8).

Table 8. Summary of the times taken to attempt to secure a prey item by juveniles at one month of age.

Data	N	Min.	Max.	Mean	SE
<i>Overall</i>	164				
Artificial incubation	130	18	592	238	13
Natural incubation	34	51	589	272	28
Female	106	18	592	234	15
Male	58	38	589	265	20
<i>Artificial inc. only</i>	130				
18°C	54	25	592	242	21
21°C	41	18	562	226	25
22°C	35	38	585	246	25
<i>Natural inc. only</i>	34				
Female	12	51	589	221	52
Male	22	79	589	300	32

Table 9. Summary of the times taken to attempt to secure a prey item by juveniles at ten months of age.

Data Categories	N	Min.	Max.	Mean	SE
<i>All juveniles</i>	183				
Artificial incubation	101	18	592	269	17
Natural incubation	82	0	581	209	16
Female	97	18	592	258	17
Male	86	0	577	224	16
<i>Artificial inc. only</i>	101				
18°C	34	18	592	243	30
21°C	34	35	569	282	28
22°C	33	59	577	283	28
<i>Natural inc. only</i>	82				
Female	30	24	581	248	29
Male	52	0	535	286	18

Table 10. Factors that affected feeding time at ten months post-hatching.

Data Categories	N	Significant Factors	F _(df)	P
All juveniles	183	Inc. treatment ¹	F ₍₁₎ = 6.65	P = 0.0107
		Jaw length	F ₍₁₎ = 10.29	P = 0.0016
		Snout-vent length	F ₍₁₎ = 9.13	P = 0.0029
		Mass	F ₍₁₎ = 10.92	P = 0.0011
Artificial inc. only	101	Jaw length	F ₍₁₎ = 6.38	P = 0.0131
		Snout-vent length	F ₍₁₎ = 5.83	P = 0.0176
		Mass	F ₍₁₎ = 8.63	P = 0.0041

¹ Inc. treatment refers to artificial versus natural incubation.

Figure 8. The time taken to respond to food as a function of juvenile size at ten months of age.

5.5. Discussion

We addressed three of our four questions investigating whether performance of juvenile tuatara varied in a way that might affect their fitness. We compared performance of juvenile tuatara with size, without confounding effects of age, as well as investigating changes in performance between one and ten months of age. We also investigated effects of incubation regime on performance because all juveniles were kept in similar conditions post-hatching. All animals had similar experience in handling and no experience of natural predators. We could not determine whether performance was directly affected by incubation temperature, as incubation temperature co-varied with sex.

5.5.1. Performance of Juvenile Tuatara

Performance can vary with size of juvenile tuatara. Longer juveniles are faster than shorter juveniles of the same age, but they also demonstrate the greatest range in sprint speeds. Longer, heavier juveniles with bigger mouths take more time to make their first attack on an item of food. However, size (length) does not affect the response of juveniles to predator stimuli.

We cannot determine whether performance is directly affected by incubation temperature. Juveniles from intermediate temperatures (21 to 23 °C cte) in natural nests are fastest but have the greatest range of speeds. However, these juveniles are also the longest of those incubated naturally. Juveniles artificially incubated at 22°C are less likely to feed at one month of age than those from other artificial treatments, and most freeze at ten months of age in response to handling. However, sex and incubation temperature co-vary in natural nests, confusing analyses of incubation temperature effects on performance. Sex may have a greater effect on performance than does incubation temperature, because 22°C produces entirely males, and 18 and 21°C produce predominantly females (98% females; Chapter 4).

Performance is affected by incubation regime (natural versus artificial incubation). Naturally incubated juveniles are the shortest animals from any incubation regime (Chapter 4), but at ten months of age these juveniles are the fastest runners. The primary response to handling is for juveniles to freeze. Hence, running responses were treated as an indication of a reaction to the predator stimuli. Freezing could be interpreted as a response to handling, or to the predator stimuli. Naturally incubated juveniles are more likely to respond to predator stimuli by running, and at ten months of

age are slightly more willing to feed and faster to feed under the test conditions than those incubated artificially.

Some aspects of performance of juvenile tuatara change over time. The primary response of one month olds to handling is to freeze. By ten months of age, having had considerably more experience in being handled, juveniles tend to vary in their response to handling. Naturally incubated juveniles and smaller juveniles are more likely to respond to handling by running. Older juveniles run faster than younger juveniles. Older juveniles are more likely to respond to predator stimuli by running.

5.5.2. Suitability of Performance Tests

Results of the willingness to feed tests indicate there may be problems with the testing technique. Fewer than half of the juveniles fed during the test period despite six days of fasting prior to the test. We do not believe six days is insufficient fasting time to elicit good feeding responses. Observations of feeding in rearing enclosures indicate juvenile tuatara are ready to feed after only two days since the last feeding episode. The times taken by juveniles to respond to food items ranged throughout the ten minute test period. The test containers were unfamiliar to the juveniles. They may require more than ten minutes to become accustomed to the surroundings and assess risks of attracting attention from predators in those surroundings by moving towards a food item. Many juveniles were seen with food items crawling over them while they remained motionless or appeared to search for an avenue of escape. These behaviours were never seen in their holding enclosures during feeding unless juveniles were already busy consuming a food item. The amount of handling experienced by juveniles during tests of willingness to feed was less than for other performance tests; handling primarily

elicits a freezing response in one month old juveniles. Other performance tests produced more varied responses to stimuli with greater amounts of handling, suggesting handling is not the reason for the lack of willingness to feed.

5.5.3. *Interpretation of Performance Results for Fitness*

Incubation temperature does not affect performance *per se*, but does affect size by ten months (Chapter 4), and longer juveniles are capable of running faster. Increasing speed with increasing size is a common performance pattern for lizards (e.g. Sinervo and Adolf 1989, Van Damme et al. 1992). Larger juvenile reptiles that sprint faster are likely to be more successful at hunting prey (Avery et al. 1982, Warner 2001), obtaining access to limited resources and a wider size range of prey items (Ferguson et al. 1982, Ferguson and Fox 1984), avoiding predation (Christian and Tracy 1981, Webb 1986), and retaining high social ranks (Garland et al. 1990). Size and performance can thus affect growth and survival, that is, life history traits that have the potential to have strong effects on lifetime reproductive success. Larger size may mean female reptiles reach maturity earlier and hence have greater relative fitness because they have opportunities to reproduce sooner and/or a longer reproductive life. Larger size may also mean female reptiles are capable of producing larger clutches once they reach maturity (Schwarzkopf and Brooks 1986, Cree 1994). Reproductive success in males of species with sexual dimorphism, where males are larger than females, may also be affected by size (Berry and Shine 1980).

The leap from performance at ten months (based on size) to interpretations for fitness of tuatara, however, is a substantial one. Tuatara may live to 100 years (Dawbin 1982), and performance may change with time (Jayne and Bennett 1990). For example, sub-

adult and juvenile tuatara run faster than adults (Pepperell 1982). Reptiles may have different performance requirements with age (Green 1988). For example, juvenile tuatara may need to be faster than adults to avoid being eaten by them. Also, larger juvenile reptiles do not always run faster (Hare 2001), suggesting factors other than speed may be important determinants of fitness. Juveniles from the natural incubation treatment are significantly shorter than those artificially incubated, in part due to substantial tail loss by ten months of age (Chapter 4), but are also the fastest group. High rates and large amounts of tail loss in the naturally incubated group indicate that artificially incubated juveniles are comparatively less aggressive in the colonial rearing conditions (Chapter 4) and this could help explain the pattern of size differences among incubation treatments. Differences in aggression are associated with incubation temperature in geckos (Flores et al. 1994). Further investigation will be required to establish the importance of performance attributes such as size and speed for fitness of tuatara, particularly with respect to tail loss. Performance ability depends on environmental context, as does fitness (Congdon et al. 1999, Irschick and Losos 1999).

Cryptic colouration and freezing responses to predator stimuli and handling may be important for juvenile tuatara survival during their first few months of life. Pine snakes cease movement and use their cryptic colouration for predator avoidance (e.g. Burger 1989). However, naturally incubated juvenile tuatara are more likely to run in response to predator stimuli than those from artificial incubation treatments, indicating artificially incubated juveniles may be at a disadvantage in the natural environment if running is required for survival.

5.5.4. Competence of Artificially Incubated Tuatara

Artificially incubated juveniles do well in captivity. They grow faster than those incubated naturally (Chapter 4). However, naturally incubated juvenile tuatara are more aggressive (Chapter 4) and faster for their size. Naturally incubated tuatara also demonstrate running responses to both aerial and ground predator stimuli suggesting this is the reaction norm for small tuatara in nature. If cryptic colouration is insufficient to protect motionless juveniles, then the results suggest artificially incubated juveniles may have lower survival in nature.

In head starting programmes, juveniles are maintained in captivity until they are large enough to be less vulnerable to gape-limited predators. It may be more appropriate to test responses to predator stimuli as a measure of when juveniles are ready for release into nature than arbitrarily waiting until juveniles reach a set size or age. This may also mean juveniles can be released earlier than the five years typical for tuatara, as changes in reactions to predators can be detected by ten months of age. More research is required to investigate effects of training or exposure to predator stimuli, performance in relation to rearing conditions (e.g. competitive versus non-competitive, natural versus artificial), and natural reaction norms for predator avoidance.

5.6. References

- Andrews, R. M., Mathies, T. and D. A. Warner. 2000. Effect of incubation temperature on morphology, growth, and survival of juvenile *Sceloporus undulatus*. *Herpetological Monographs* 14:420-431.
- Avery, R. A., Bedford, J. D. and C. P. Newcombe. 1982. The role of thermoregulation in lizard biology: predator efficiency in a temperate diurnal basker. *Behavioural Ecology and Sociobiology* 11:261-267.
- Benton, M. J. 1990. Vertebrate Palaeontology: Biology and Evolution. HarperCollins Academic, London.
- Berry, J. F. and R. Shine. 1980. Sexual size dimorphism and sexual selection in turtles (order Testudines). *Oecologia* 44:185-191.
- Burger, J. 1989. Incubation temperature has long-term effects on behaviour of young pine snakes (*Pituophis melanoleucus*). *Behavioural Ecology and Sociobiology* 24:201-207.
- Burger, J. 1990. Effects of incubation temperature on behaviour of young black racers (*Coluber constrictor*) and kingsnakes (*Lampropeltis getulus*). *Journal of Herpetology* 24:158-163.
- Chiszar, D., Smith, H. M. and C. W. Radcliffe. 1993. Zoo and laboratory experiments on the behaviour of snakes: assessments of competence in captive-raised animals. *American Zoologist* 33:109-116.
- Christian, K. A. and C. R. Tracy. 1981. The effect of the thermal environment on the ability of hatchling Galapagos land iguanas to avoid predators during dispersal. *Oecologia* 49:218-223.
- Congdon, J. D., Nagle, R. D., Dunham, A. E., Beck, C. W., Kiney, O. M. and S. R. Yeomans. 1999. The relationship of body size to survivorship of hatchling snapping turtles (*Chelydra serpentina*): an evaluation of the "bigger is better" hypothesis. *Oecologia* 121:224-235.
- Cree, A. 1994. Low annual reproductive output in female reptiles from New Zealand. *New Zealand Journal of Zoology* 21:351-372.
- Cree, A. and D. Butler. 1993. Tuatara Recovery Plan (*Sphenodon* spp.). Threatened Species Recovery Plan Series No. 9. Department of Conservation, Wellington, New Zealand.
- Cree A., Thompson, M. B. and C. H. Daugherty. 1995. Tuatara sex determination. *Nature* 375:543.

- Daugherty, C. H. 1998. From dark days to a bright future: survival of the tuataras. *ZooNooz* April:8-13.
- Daugherty, C. H. and A. Cree. 1990. Tuatara, a survivor from the dinosaur age. *New Zealand Geographic* 6:66-86.
- Dawbin, W. H. 1982. The tuatara *Sphenodon punctatus*: aspects of life history, growth and longevity. Pp. 237-250. In Newman, D. G. (Ed.). *New Zealand Herpetology*. New Zealand Wildlife Service Occasional Publication No. 2.
- Downs, S. J. and R. Shine. 1999. Do incubation-induced changes in a lizard's phenotype influence its vulnerability to predators? *Oecologia* 120:9-18.
- Elphick, M. J. and R. Shine. 1998. Longterm effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards (*Bassiana duperreyi*, Scincidae). *Biological Journal of the Linnean Society* 63:429-447.
- Elphick, M. J. and R. Shine. 1999. Sex differences in optimal incubation temperature in a scincid lizard species. *Oecologia* 118:431-437.
- Falla, R. A., Sibson, R. B. and E. G. Turbott. 1978. *Collins Guide to the Birds of New Zealand and Outlying Islands*. Harper Collins Publishers, Auckland, New Zealand.
- Ferguson, G. W., Brown, K. L. and V. G. DeMarco. 1982. Selective basis for the evolution of variable egg and hatchling size in some iguanid lizards. *Herpetologica* 38:178-188.
- Ferguson, G. W. and S. F. Fox. 1984. Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. *Evolution* 38:342-349.
- Flores, D., Tousignant, A. and D. Crews. 1994. Incubation temperature affects the behaviour of adult leopard geckos (*Eublepharis macularius*). *Physiology and Behaviour* 55:1067-1072.
- Garland, T. Jr., Hankins, E. and R. B. Huey. 1990. Locomotor capacity and social dominance in male lizards. *Functional Ecology* 4:243-250.
- Green, H. W. 1988. Antipredator mechanisms in reptiles. Pp. 1-52. In Gans, C. and R. B. Huey (Eds.). *Biology of the Reptilia*, Vol 16. Liss, New York.
- Hare, K. M. 2001. Effects of Constant Incubation Regimes on Eggs and Hatchlings of the Egg-laying Skink, *Oligosoma suteri*. Unpublished MSc Thesis. Victoria University of Wellington, New Zealand.
- Irschick, D. J. and J. B. Losos. 1999. Do lizards avoid habitats in which performance is submaximal? The relationship between sprinting capabilities and structural habitat use in Caribbean Anoles. *The American Naturalist* 154:293-305.

- Jayne, B. C. and A. F. Bennett. 1990. Selection on locomotion performance capacity in a natural population of garter snakes. *Evolution* 44:1204-1229.
- Lima, S. L. and L. M. Dill. 1990. Behavioural decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* 68:619-640.
- Nelson, N. J. 1998. Conservation of Brothers Island Tuatara, *Sphenodon guntheri*. Unpublished MConSc Thesis. Victoria University of Wellington, New Zealand.
- Pepperell, J. G. 1982. Tuatara *Sphenodon punctatus* locomotion: a summary. Pp. 207-211. In Newman, D. G. (Ed.). New Zealand Herpetology. New Zealand Wildlife Service Occasional Publication No. 2.
- Qualls, C. P. and R. M. Andrews. 1999. Cold climates and the evolution of viviparity in reptiles: cold incubation temperatures produce poor quality offspring. *Biological Journal of the Linnean Society* 67:353-376.
- Rhen, T. and J. W. Lang. 1995. Phenotypic plasticity for growth in the common snapping turtle: effects of incubation temperature, clutch, and their interaction. *The American Naturalist* 146: 726-747.
- Ridley, M. 1997. Evolution. Oxford University Press. Oxford.
- Schwarzkopf, L. and R. J. Brooks. 1986. Annual variation in reproductive characteristics of painted turtles (*Chrysemys picta*). *Canadian Journal of Zoology* 64:1148-1151.
- Self, S. G. and K. -Y. Liang. 1987. Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under non-standard conditions. *Journal of the American Statistical Association* 82:605-610.
- Shine, R., Elphick, M. J. and P. S. Harlow. 1997. The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology* 78:2559-2568.
- Sinervo, B. and S. C. Adolf. 1989. Thermal sensitivity of growth rate in hatchling *Sceloporus* lizards: environmental, behavioural and genetic aspects. *Oecologia* 78:411-419.
- Stearns, S. C. and J. C. Koella. 1986. The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. *Evolution* 40:893-913.
- Van Damme, R., Bauwens, D., Brana, F. and R. F. Verheyen. 1992. Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. *Herpetologica* 48: 220-228.
- Warner, D. A. 2001. Phenotypes and Survival of Hatchling Lizards. Unpublished MSc Thesis. Virginia Polytechnic Institute and State University.

- Webb, P. V. 1986. Locomotion and predator-prey relationships. Pp 24-41. In Feder, M. E. and G. V. Lauder (Eds.). *Predator-prey Relationships: Perspectives and Approaches from the Study of Lower Vertebrates*. University of Chicago Press, Chicago.

Chapter 6. Quality control: are artificially incubated tuatara up to the standard set by natural incubation?

6.1. Introduction

Since 1989, tuatara eggs have been incubated in constant artificial conditions to produce founders for new captive and wild populations, and to augment existing wild populations (Daugherty 1998, Nelson 1998). In addition, eggs have been collected by another artificial procedure, induction of oviposition. Tuatara have temperature-dependent sex determination (TSD; Cree et al. 1995, Chapter 4). Incubation temperatures during embryonic development affect size and performance of reptiles, including tuatara (e.g. Van Damme et al. 1992, this thesis), and phenotypic differences between individuals can affect relative biological fitness (Ridley 1997). I review the evidence collected in Chapters 2 to 5 for whether artificially induced and incubated tuatara meet the quality standards set by natural incubation. Recommendations for future induction of oviposition, artificial incubation and research are made based on this review.

6.2. Review of Results: Chapters 2-5

6.2.1. Induction of Oviposition

- Induction of oviposition produces smaller eggs.
- Smaller eggs result in smaller juveniles, and these effects are still apparent when juveniles are 10 months old.

- Initial egg mass is the most important predictor of hatchling size.
- Induction does not require knowledge of nesting season or timing.

6.2.2. Incubation Regime

- Hatching success is higher when eggs are artificially incubated, than in more variable natural nest conditions.
- Incubation duration is shorter in artificial conditions compared to in natural nests.
- Sex ratio of hatchlings can be reliably manipulated using artificial incubation.
- The pivotal sex-determining temperature for tuatara from Stephens Island lies between 21 and 22°C.
- Artificial incubation temperatures do not affect size at hatching, but by ten months of age, hatchlings from 18°C are significantly smaller than those from other artificial regimes.
- Artificially incubated juveniles are larger by ten months of age than those from natural incubation regimes.
- Naturally incubated juveniles are the fastest runners at ten months of age, despite being the shortest animals from any incubation treatment.
- Naturally incubated juveniles are more likely to respond to predator stimuli by running than those incubated artificially.
- Naturally incubated juveniles are slightly more willing to feed and faster to feed under the test conditions at ten months of age than those incubated artificially.
- Naturally incubated juveniles may be more aggressive than artificially incubated juveniles, based on tail loss data, potentially driving size and performance patterns.

6.3. Discussion

Induction produces smaller tuatara eggs than those laid in natural nests. Smaller eggs result in smaller juveniles. However, eggs can be collected more efficiently by induction than by collection from natural nests, which relies on finding natural nests and knowledge of the nesting season. Incubation conditions do not significantly affect size at hatching (within the conditions tested), but by ten months of age, artificially incubated hatchlings are larger than those incubated naturally. Mortality in natural nests is higher than in controlled artificial conditions. Juvenile mortality during the ten month study period in the captive situation was insignificant from both natural and artificial incubation regimes. Hence, more juveniles of larger size are produced by artificial incubation, despite whether eggs are collected naturally or by induction.

Bigger, however, does not necessarily mean better. Naturally incubated juveniles are the shortest animals from any incubation regime, but at ten months of age these juveniles are the fastest runners. Also, naturally incubated juveniles are more likely to respond to predator stimuli by running, and at ten months of age are slightly more willing to feed and faster to feed under the test conditions than those incubated artificially. Artificially incubated juvenile tuatara appear to be less aggressive. Together these results suggest artificially incubated juveniles may have lower survival in nature and hence reduced fitness compared to those incubated naturally.

Therefore, artificial incubation is a procedure that benefits conservation as it can be used reliably to produce founders for new populations. Induction is not the preferred method for egg collection, especially where time and knowledge allow eggs to be

collected from natural nests. Of the artificial incubation conditions, 18°C is predominantly used to produce female tuatara. Juveniles from this treatment are significantly smaller by ten months of age than females produced at 21°C, or males produced at 22°C. Future incubation for females could be conducted at, say, 20°C. This is likely to produce juveniles of larger size than those from 18°C, while maintaining a buffer of more than 1°C from the pivotal temperature, to ensure production of females. Effects on performance of artificial incubation regimes that mimic temperatures in natural nests warrant investigation. Further research is required to establish the relevance of performance test results in nature and consequences in the longer term for relative fitness of juveniles from artificial versus natural incubation regimes.

Sex ratios of juveniles produced under constant incubation conditions in this thesis vary slightly from those documented by Cree et al. (1995). I propose this is likely to be due to increased reliability of equipment and sophistication of temperature recording since their study. Unfortunately, the slight differences in sex ratios between studies resulted, unexpectedly, in the inability to distinguish between effects of incubation temperature and sex on performance of juveniles from artificial incubation conditions.

6.4. Recommendations for Future Research

Research that would help to further evaluate quality control issues of artificial procedures for production of threatened reptiles with TSD include the following questions:

1. Do incubation induced size and performance differences last long enough to be biologically meaningful in terms of fitness?

Notes: For tuatara from this study, this question needs to be addressed over the next two decades, that is, at least until tuatara from test incubation conditions kept in similar rearing conditions reach sexual maturity.

2. What affects post-hatching growth, and how is this influenced by incubation conditions?

Notes: Approaches to this question could include social aspects of rearing conditions, including behavioural differences between individuals such as wariness or aggression, and temperature selection of individuals based on incubation conditions. Rearing juveniles in individual enclosures is recommended in the future.

3. Does performance (e.g. sprint speed, predator avoidance) vary with rearing conditions?

Notes: Juvenile mortality of reptiles is believed to be high in nature, hence head-starting is viewed as a good method of increasing survival of founders for new populations.

Head-started juvenile tuatara have been released into nature and appear to be doing well (Nelson 1998). However, performance comparisons of animals reared in artificial conditions and in nature have not been conducted.

4. Are apparent differences in aggression between artificially and naturally incubated juveniles significant in terms of fitness?

Notes: Communal rearing conditions experienced by juveniles in this study potentially exacerbated aggressive encounters. Tail loss in reptiles represents a loss of resources.

Is this loss of resources offset by a survival advantage due to aggression, for example?

Is tail loss affected by the sex ratio of hatchlings in enclosures? How does tail loss affect growth of hatchlings?

5. Do juveniles from variable artificial incubation regimes have similar performance characteristics to those incubated in natural nests?

Notes: That is, should artificial incubation of tuatara eggs be conducted at variable temperatures similar to natural conditions instead of constant temperatures?

Other avenues of research that warrant investigation:

6. How do performance test results change if conducted at night?

Notes: Juvenile tuatara are active during the day and night, but adult tuatara are mainly nocturnal.

7. Does the relationship between female snout-vent length and clutch size explain variation in clutch size observed in chapter 2 between induced and naturally laid clutches?

8. Do substrate or ambient temperature affect adult female tuatara nest site selection?

9. Does water availability in the oviduct and during incubation affect size and/or performance of juvenile tuatara? Also, does induction affect performance other than through effects on size?

Notes: Effects of water availability were not investigated in this thesis. Eggs in natural nests were exposed to variable water conditions, while artificially incubated eggs were maintained at a constant water potential.

10. Do tuatara have a second pivotal sex determining temperature?

Notes: Incubation at constant temperatures above 22°C should be attempted. Evidence presented in this thesis suggests a second pivotal temperature does not exist and that the sex determining pattern for tuatara is female-male, that is, females are produced at cooler temperatures than males. This is based on the pattern in natural nests, where incubation temperatures reached 34°C, and the warmest nests still produced males.

Cree et al. (1995) propose the sex determining pattern for *S. guntheri* is female-male-female, but they used mean temperature for a variable incubation regime as support for the second pivotal temperature, when actual incubation temperatures during the middle period of incubation (22-23°C) or constant temperature equivalent (as used in Chapter 3) would probably have been more appropriate. I suggest the sex determining pattern for *S. guntheri* is also female-male.

6.5. References

- Cree A., Thompson, M. B. and C. H. Daugherty. 1995. Tuatara sex determination. *Nature* 375:543.
- Daugherty, C. H. 1998. From dark days to a bright future: survival of the tuataras. *ZooNooz* April:8-13.
- Nelson, N. J. 1998. Conservation of Brothers Island Tuatara, *Sphenodon guntheri*. Unpublished MConSc Thesis. Victoria University of Wellington, New Zealand.
- Ridley, M. 1997. Evolution. Oxford University Press. Oxford.
- Van Damme, R., Bauwens, D., Brana, F. and R. F. Verheyen. 1992. Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. *Herpetologica* 48: 220-228.