

# Egg mass determines hatchling size, and incubation temperature influences post-hatching growth, of tuatara *Sphenodon punctatus*

Nicola J. Nelson<sup>1\*</sup>, Michael B. Thompson<sup>2</sup>, Shirley Pledger<sup>3</sup>, Susan N. Keall<sup>1</sup> and Charles H. Daugherty<sup>1</sup>

<sup>1</sup> School of Biological Sciences, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand

<sup>2</sup> School of Biological Sciences and Wildlife Research Institute, Heydon-Laurence Building (A08), University of Sydney, NSW 2006, Australia

<sup>3</sup> School of Mathematics and Computing Sciences, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand

(Accepted 6 November 2003)

## Abstract

The size of reptile hatchlings can be phenotypically plastic in response to incubation temperature, and size is a trait likely to influence fitness – i.e. hatchling size is proposed as an indicator of quality. The parental and incubation temperature effects on the size of one of New Zealand's most biologically significant reptile species, the tuatara *Sphenodon punctatus* are investigated. Artificial incubation at constant temperatures is used to produce founders for new captive and wild populations of tuatara and to augment existing rare populations. We compare size of hatchling tuatara from artificial and natural incubation treatments. The relationship of hatchling size with incubation temperature and sex is examined, and we investigate whether our results support differential fitness models for the evolution of temperature-dependent sex determination in tuatara. Initial egg mass is the most important factor affecting size of hatchling tuatara and is still an important influence at 10 months of age. Incubation temperature does not greatly influence size of hatchlings, but significantly influences size by 10 months of age. Constant artificial incubation conditions result in larger, but possibly less aggressive, juveniles than those from more variable natural incubation conditions by 10 months of age. Evidence from size patterns of tuatara incubated in natural nests supports differential fitness models for the adaptive significance of temperature-dependent sex determination. Thermal variation has little effect on size of male hatchlings, but female embryos that develop in more stable thermal conditions, in more reliable sites for hatching, are bigger and have longer jaws.

**Key words:** *Sphenodon punctatus*, tuatara, reptile, incubation, size

## INTRODUCTION

Few studies of reptiles have assessed persistence of incubation effects past hatching, or the biological significance of such effects, despite the presumption that such effects are likely to influence fitness (Elphick & Shine, 1998; Shine, 1999). Incubation temperature, for example, affects many aspects of hatchling phenotype in reptiles, including size, shape, colour, sex, locomotor performance, metabolism, growth rates and survival (e.g. Gutzke & Packard, 1987; Joanen, McNease & Ferguson, 1987; Burger, 1990; Brooks *et al.*, 1991; Deeming & Ferguson, 1991; Van Damme *et al.*, 1992; Spotila *et al.*, 1994; Cree, Thompson & Daugherty, 1995; Elphick & Shine, 1998). The prevailing view is that hatchling size is an indicator of quality (Head, May & Pendleton, 1987; Miller, Packard & Packard, 1987; Packard & Packard, 1988). For example, in the short term, larger juvenile side-blotched lizards *Uta stansburiana* 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 & Fox, 1984). In the longer term, the 'bigger is better' hypothesis implies that smaller reptiles may not be as successful at maintaining territories or achieving mating opportunities (Froese & Burghardt, 1974; Swingland & Coe, 1979; Berry & Shine, 1980; Webb *et al.*, 1987; Lang, Andrews & Whitaker, 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).

We investigate the effect of incubation temperature on size of tuatara *Sphenodon punctatus* hatchlings, and whether the influence is still apparent at 10 months post-hatching. We also examine the relationship of hatchling size with incubation temperature and sex, and investigate whether our results support differential fitness models for the evolution of temperature-dependent sex determination (TSD) in tuatara. Tuatara are medium-sized, sexually dimorphic reptiles (Dawbin, 1982; Cree *et al.*, 1995). Now restricted to offshore islands of New Zealand, they are

\*All correspondence to: Nicola J. Nelson.  
E-mail: nicola.nelson@vuw.ac.nz

of high conservation importance (Cree & Butler, 1993; IUCN, 1996) and are biologically significant as the sole living representatives of the reptilian order Sphenodontia (Benton, 1990; Cree & Butler, 1993).

Eggs have been collected and artificially incubated to produce founders for new captive and wild populations and to augment existing rare populations of tuatara (Daugherty, 1998; Nelson, 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). Despite analysis of the effects of constant incubation temperatures on tuatara eggs up to the time of hatching (Thompson, 1990), the biological significance and persistence of effects of incubation environments on fitness and lifetime reproductive success can only be ascertained in the longer term, as is true for all species.

Analyses of the effects of incubation 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, Elphick & Harlow, 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 and sex effects are confounded in many experimental designs resulting from the very nature of TSD (but see Rhen & 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 parental genetic aspects (Schwarzkopf & Brooks, 1987; Cagle *et al.*, 1993; Rhen & Lang, 1995; Olsson *et al.*, 1996; Shine & Harlow, 1996; Sinervo & Doughty, 1996; Reinhold, 1998; Steyermark & Spotila, 2001). Parental factors are not always included in analyses.

We compare size of artificially incubated hatchlings and juveniles with those incubated in natural nests, during the same season. Our rationale was to determine which constant temperature incubation regime most closely approximates the optimal temperature for producing hatchlings for reintroduction into the wild.

## MATERIALS AND METHODS

Eggs of tuatara from a single laying season were incubated in 2 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 and 21 November 1998, 320 eggs were

collected using 2 methods: induction of oviposition by injection of synthetic oxytocin (Oxytocin-s, conc. 10 IU/ml, Intervet International BV, Boxmeer, Holland;  $n = 166$  eggs; 21 clutches), and recovery of eggs laid naturally in nests ( $n = 154$  eggs; 29 clutches). November is within the natural egg-laying season for tuatara on Stephens Island (Thompson, 1990; Thompson, Newman & Watson, 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 ( $\sim -170$  kPa) at room temperature on the island ( $\sim 18$ – $22$  °C). On 21 November 1998, the eggs were flown by helicopter to Victoria University of Wellington for incubation.

Each incubation box (sealed 2-l plastic container half-filled with moistened vermiculite ( $-170$  kPa; 120 g medium grade vermiculite to 96 ml water per box)) contained 4 induced and 4 naturally laid eggs. Clutches were divided equally into 3 groups, and each group was randomly assigned to 1 of 3 constant incubation temperatures (18, 21 or 22 °C). Eggs were then randomly assigned to an incubation box within each treatment. No clutch was represented by more than 1 egg per incubation box. 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  $\sim -170$  kPa. Incubators were calibrated prior to use, and incubation temperatures were checked daily. Temperatures were recorded by dataloggers placed at the centre of the middle shelf of the incubator (waterproof Stowaway® Tidbit® temperature recorders, dimensions: 30 × 41 × 17 mm, Onset Computer Corporation, Massachusetts, U.S.A.; hourly recordings). Incubation boxes were moved within each incubation chamber daily (Lee & Rawlings, 1982).

During the period when eggs were collected for artificial incubation, 25 naturally laid nests were selected and marked for natural incubation. Selected nests encompassed a diversity of nest characteristics including location, nest depth and soil type. Where more than 1 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 datalogger 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 × 200 mm square of wire mesh (mesh size 10 × 10 mm) to protect them from excavation by other nesting females.

After approx. 11 months, the natural incubation period (McIntyre, 1997), the nests were excavated, and unhatched eggs and hatchlings were collected and taken to Victoria University of Wellington, New Zealand. All eggs were numbered and placed in incubation boxes as for the

artificially incubated eggs, and incubated at 22 °C until hatching. Dataloggers 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 to provide 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–posterior end of jaw on right side)), and individually marked with a toe-clip<sup>1</sup> on the day they hatched, and subsequently weighed and measured monthly (except at 2 months) until 10 months of age. Juvenile tuatara cannot be sexed externally with confidence until they attain ~130–160 mm SVL (A. Cree pers. comm.), so laparoscopy of internal reproductive organs was conducted at about 1 year after hatching to ascertain the sex of juveniles.

Hatchlings were reared in similar conditions, with 16 individuals per 600 × 700 mm enclosure, grouped according to hatch date. Enclosures were furnished with a leaf litter base, tree bark for shelter, and 2 water dishes. Juveniles were fed 3 times per week by releasing similar amounts of invertebrates into each enclosure regardless of juvenile size (e.g. ~50 house flies per feeding episode per enclosure). Prey items were generally provided in excess of what juveniles were capable of eating immediately, but between feeding episodes there was potential for competition for remaining food items. Enclosures were exposed to daily temperature variations of between ~18–22 °C and a 12 : 12 light/dark cycle beginning at 06:00 h throughout the year (Duro-test® True-lite® power twist fluorescent tubes, placed 750 mm above the enclosure).

### Statistical analyses

Size of juveniles was analysed using multivariate analysis of variance (MANOVA). Data for juveniles resulting from artificial and natural incubation were analysed separately. Dependent variables included in analyses of artificially incubated young were jaw length, jaw width, SVL, total length and mass at hatching or 10 months of age. Fixed factors were incubation temperature or sex. These factors were never included together as incubation temperature co-varies with sex (TSD). Parental factors investigated were clutch as a random effect and initial egg mass as a covariate. We analysed 3 sets of data from artificially incubated young. The first contained all artificially incubated juveniles ( $n = 278$  for all measurements except mass at hatching;  $n = 277$  for mass at hatching). The second was female hatchlings (all juveniles resulting from 18 and 21 °C artificial incubation treatments, minus 3 males produced at 21 °C ( $n = 170$ )), hence controlling for any influence of sex on size. The third data set included only females from the 21 °C treatment and males from

the 22 °C treatment, i.e. where incubation temperature was controlled for as much as possible ( $\pm 1$  °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. Only individuals for which complete records were available were included in these analyses.

Size of naturally incubated young was analysed using sex as a fixed factor, and incubation temperature or clutch identity as random factors. The 2 random factors were not included together as in most cases clutch identity represented the same information as the temperature variable. The variables used to represent incubation temperatures in natural nests were mean temperature throughout incubation, variance (standard deviation<sup>2</sup>) in temperature throughout incubation, duration of incubation, or mean constant temperature equivalent (CTE) in February. Mean CTE 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, Limpus & Stoutjesdijk, 1994). For tuatara, males are predicted to be produced if the CTE of a nest exceeds the threshold temperature for sex determination of males in artificial conditions; females are predicted to be produced from nests where the CTE is lower than the threshold. For each nest, mean CTE during February was calculated from hourly temperature records using Georges' model (Georges, 1989; Georges *et al.*, 1994) by assuming that the developmental zero is 11.1 °C (based on incubation duration at constant temperatures and assuming a linear relationship below 18 °C), the reference temperature is 18 °C with an incubation period of 264 days, and the pivotal temperature is 21 °C (Nelson *et al.*, in press). Temperatures during February were selected because February was assumed to include the thermo-sensitive period for sex determination. Tuatara eggs are laid in November and reach the developmental stage before hatching, just prior to winter (around May; Dendy, 1899). We do not know when the thermo-sensitive period for sex determination is in tuatara, but assume that they follow a similar temporal pattern to other reptiles, i.e. that sex determination occurs during the middle third of development (Yntema, 1979; Bull & Vogt, 1981).

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 10 months from 21 clutches).

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. Hypothesis test results are not generally reported because of: (1) the large number

<sup>1</sup> The Ethical Committee of the Zoological Society of London considers that toe-clipping is no longer acceptable as a routine procedure for marking animals.

**Table 1.** Summary of measurements of tuatara *Sphenodon punctatus* at hatching and 10 months from artificial (18, 21 and 22 °C) and variable natural incubation temperatures. <sup>a</sup>, variable temperatures; <sup>b</sup>, *n* = 136 at hatching, *n* = 135 at 10 months of age

	22 °C				21 °C				18 °C				Natural nests <sup>a,b</sup>			
	Mean	SE	Min.	Max.	Mean	SE	Min.	Max.	Mean	SE	Min.	Max.	Mean	SE	Min.	Max.
	<i>n</i> = 110				<i>n</i> = 79				<i>n</i> = 105							
<i>Jaw length (mm)</i>																
Hatching	16.4	0.07	13.1	17.8	16.2	0.09	12.1	17.6	15.9	0.07	14.3	17.1	15.7	0.05	13.5	17.4
10 months	22.9	0.08	20.9	24.8	23.2	0.10	19.5	25.1	21.9	0.10	17.9	24.7	21.0	0.10	17.2	23.8
<i>Jaw width (mm)</i>																
Hatching	11.2	0.06	8.9	12.4	11.4	0.05	10.2	12.4	11.3	0.05	10.0	12.2	11	0.04	9.4	12.0
10 months	16.3	0.08	11.9	18.4	16.7	0.08	14.9	18.1	15.7	0.08	13.0	17.7	15	0.08	12.7	18.8
<i>SVL (mm)</i>																
Hatching	53	0.23	45	58	53	0.25	48	58	53	0.25	46	59	53	0.18	46	58
10 months	84	0.39	64	95	86	0.40	77	93	80	0.43	64	92	76	0.45	62	88
<i>Total length (mm)</i>																
Hatching	107	0.52	90	121	108	0.50	95	117	105	0.54	89	118	106	0.44	92	118
10 months	163	1.58	93	199	167	1.68	127	192	153	1.43	98	192	125	1.61	76	175
<i>Mass (g)</i>																
Hatching	4.55	0.06	2.81	5.78	4.70	0.07	3.02	5.92	4.65	0.06	3.10	5.91	4.59	0.06	2.80	6.00
10 months	17.65	0.25	6.77	28.48	18.08	0.30	12.50	23.78	14.05	0.23	6.50	21.47	12.25	0.22	5.95	21.30

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 & Anderson, 1998) for each combination or model. We used the small-sample version of the AIC equation (termed AIC<sub>c</sub>) designed for cases where there are relatively few data per estimated parameter (Burnham & Anderson, 1998). AIC<sub>c</sub>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<sub>c</sub>).

Incubation box effects were not significant and were left out of all analyses. Enclosure effects could not be allowed for because of the limitations imposed on the experimental design by highly variable hatching dates among temperature treatments. All means of measurements are presented  $\pm 1$  standard error (SE). All significant factors determined by MANOVA and MANCOVA analyses are underlined ( $P < 0.05$ ).

## RESULTS

Incubation took on average  $165 \pm 0.54$  days at constant 22 °C ( $n = 113$ ),  $183 \pm 0.60$  days at 21 °C ( $n = 80$ ) and  $264 \pm 2.03$  days at 18 °C ( $n = 105$ ). At variable temperatures in natural nests, incubation took on average  $365 \pm 1.92$  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.

Mean SVL of hatchling tuatara over all treatments was 53 mm (range 45–59 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 10 months, all juveniles had increased in size for all measurements (Table 1).

Incubation temperature determined sex of hatchlings, with 100% females produced at 18 °C, mostly females at 21 °C (96%), and 100% males at 22 °C (Table 2). The pivotal temperature lies between 21 and 22 °C.

### Effects of artificial incubation on tuatara size

Parental factors were the most important influences on size of hatchling tuatara. Hatchlings from larger eggs were longer, and this pattern was retained until 10 months post-hatching (N. J. Nelson, pers. obs). Incubation temperature/sex influenced size of hatchlings through interaction terms with clutch identity or initial egg mass in multivariate analyses (Table 3a). Otherwise, common suites of influences over all individual measurements of size were not apparent (Table 3b).

Juveniles incubated at 21 °C were larger than any other treatment group in all measurements after 6 months of age (Fig. 1). These females were on average larger than males incubated at 22 °C by 10 months of age,

**Table 2.** Effects of artificial incubation temperature on sex. Tuatara *Sphenodon punctatus* have temperature-dependent sex determination with the pivotal temperature between 21 and 22 °C

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

**Table 3.** Incubation temperature and parental effects on size of artificially incubated tuatara *Sphenodon punctatus*. Influences investigated include 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. Terms were added as long as the Wilk's lambda test had  $P < 0.05$ . <sup>a</sup>, where sex is listed, it could equally read incubation temperature, as results are identical

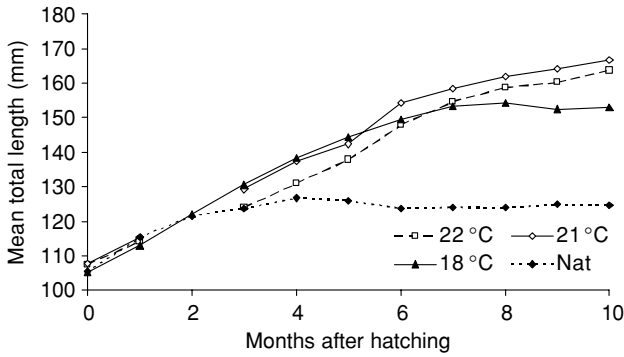
Data set	Best approximating models	<i>n</i>
All juveniles at hatching	it + clutch + iem + it * clutch + iem * it + iem * clutch + iem * it * clutch <i>or</i> sex + clutch + iem + sex * clutch + iem * sex + iem * clutch + sex * clutch * iem	278
All juveniles at 10 months	it + clutch + iem + it * clutch + iem * it + iem * clutch + iem * it * clutch	278
Females only at hatching	it + clutch + iem + it * clutch + iem * it + iem * clutch + iem * it * clutch	170
Females only at 10 months	it + clutch + iem + it * clutch + iem * it + iem * clutch + iem * it * clutch	170
21 °C females + 22 °C males at hatching <sup>a</sup>	sex + clutch + iem + sex * clutch + iem * sex + iem * clutch + iem * sex * clutch	176
21 °C females + 22 °C males at 10 months <sup>a</sup>	sex + clutch + iem + sex * clutch + iem * sex + iem * clutch + iem * sex * clutch	176

(b) Models that best represent each size trait respectively from general linear model procedure results ranked by Akaike information criteria (AIC<sub>c</sub>). All models within four of the lowest AIC<sub>c</sub> are listed. The most important term defined by the level of significance in ANOVAs is underlined. *K* refers to the bias adjustment term and provides a guide to the number of parameters in the AIC model

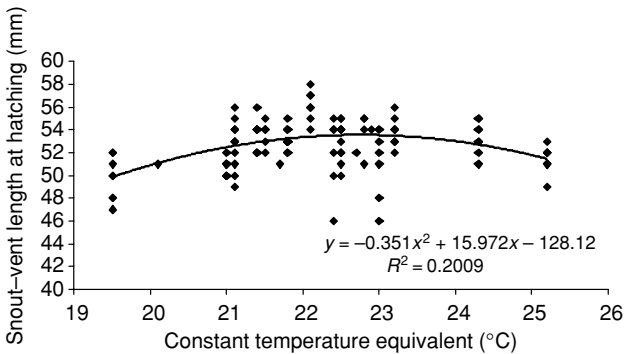
Data set	Best approximating models	<i>K</i>	Best approximating models	<i>K</i>
<i>All juveniles at hatching (n = 277)</i>			<i>At 10 months (n = 277)</i>	
Jaw length	it + clutch + <u>iem</u> + it * iem <i>or</i> sex + clutch + <u>iem</u> + sex * iem <i>or</i> it + clutch + <u>iem</u>	55 53 53	<u>it</u> + iem + it * iem	7
Jaw width	clutch + <u>iem</u>	51	<u>it</u> + iem + it * iem	7
SVL	it + clutch + <u>iem</u> + it * iem <i>or</i> it + clutch + <u>iem</u>	55 53	<u>it</u> + iem + it * iem	7
Total length	it + clutch + <u>iem</u>	53	<u>it</u> + iem + it * iem	7
Weight	it + clutch + <u>iem</u> + it * iem <i>or</i> sex + clutch + <u>iem</u> + sex * iem	55 53	<u>it</u> + iem + it * iem	7
<i>Females only at hatching (n = 170)</i>			<i>At 10 months (n = 170)</i>	
Jaw length	it + <u>iem</u> + it * iem <i>or</i> it + <u>iem</u>	5 4	<u>it</u> + clutch + iem + it * iem	52
Jaw width	it + clutch + <u>iem</u>	51	<u>it</u> + iem + it * iem <i>or</i> <u>it</u> + iem	5 4
SVL	it + clutch + <u>iem</u> + it * iem <i>or</i> it + <u>iem</u> + it * iem <i>or</i> it + clutch + <u>iem</u> <i>or</i> it + <u>iem</u>	52 5 51 4	<u>it</u> + iem + it * iem <i>or</i> <u>it</u> + iem	5 4
Total length	it + clutch + <u>iem</u> + it * iem <i>or</i> it + clutch + <u>iem</u>	52 51	it + <u>iem</u> + it * iem <i>or</i> it + <u>iem</u>	5 4
Mass	it + clutch + <u>iem</u> + it * iem <i>or</i> it + clutch + <u>iem</u> <i>or</i> clutch + <u>iem</u>	52 51 50	<u>it</u> + iem + it * iem <i>or</i> <u>it</u> + iem	5 4
<i>21 °C females + 22 °C males at hatching (n = 176)</i>			<i>At 10 months (n = 176)</i>	
Jaw length	sex + <u>iem</u> + sex * iem <i>or</i> sex + <u>iem</u>	5 4	sex + <u>iem</u> + sex * iem <i>or</i> sex + <u>iem</u>	5 4
Jaw width	sex + <u>iem</u> + sex * iem <i>or</i> sex + <u>iem</u> <i>or</i> <u>iem</u>	5 4 3	sex + <u>iem</u> + sex * iem <i>or</i> sex + <u>iem</u>	5 4
SVL	sex + <u>iem</u> + sex * iem <i>or</i> sex + clutch + <u>iem</u> <i>or</i> sex + <u>iem</u> <i>or</i> <u>iem</u>	5 49 4 3	sex + <u>iem</u> + sex * iem <i>or</i> sex + <u>iem</u>	5 4
Total length	clutch + <u>iem</u>	48	sex + <u>iem</u> + sex * iem	5
Mass	sex + <u>iem</u> + sex * iem	50	sex + <u>iem</u> + sex * iem <i>or</i> sex + <u>iem</u> <i>or</i> <u>iem</u>	5 4 3

indicating either sexual dimorphism is already subtly apparent, or 1 °C is sufficient to influence the size of these traits (Table 1, Fig. 1). The interpretation of early detection of sexual dimorphism is unlikely, as the

pattern is opposite to what would be expected, i.e. adult females are smaller in length and in head size than adult males, not larger (Dawbin, 1982). Differences in size (SVL) were not explained by differences in incubation



**Fig. 1.** Total length of tuatara *Sphenodon punctatus* to 10 months post-hatching by incubation treatment (constant artificial temperatures 18, 21 or 22 °C; variable natural temperatures (Nat)). Juveniles incubated at 21 °C were larger than any other incubation treatment group after 6 months of age.



**Fig. 2.** The influence of incubation temperature on size of naturally incubated tuatara *Sphenodon punctatus* hatchlings. Hatchling SVL was maximized in natural nests with the constant temperature equivalent (CTE) between 21 and 23 °C.

duration due to incubation temperature ( $F_{1,276} = 0.01$ ,  $P = 0.9142$ ). However, within the 21 and 22 °C treatments respectively, eggs that incubated longer produced significantly longer hatchlings ( $F_{1,72} = 8.88$ ,  $P = 0.0039$ ;  $F_{1,103} = 48.46$ ,  $P = 0.0001$  respectively). Hatchlings from 18 °C had smaller jaw lengths and total lengths than those from other artificial treatments, and this effect was still apparent at 10 months (Tables 1 and 3b).

**Natural incubation and differential size with incubation conditions**

Size of naturally incubated hatchlings was affected by incubation temperature and this effect persisted until juveniles were at least 10 months of age (Table 4). Hatchling SVL was maximized in natural nests with the CTE between 21 and 23 °C (Fig. 2). Females hatched from cooler nests, and embryos in cooler nests took longer to develop. Females that incubated for longer were smaller at 10 months, but this pattern was not apparent for males (Fig. 3). Females from nests with greater temperature variance had smaller jaw lengths by 10 months, than those from nests with smaller temperature variance. Males hatched from nests with greater variance in temperature

**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 (CTE) in February

(a) Models that best represent size data including jaw length, jaw width, SVL, total length (mm) and mass (g) using the MANOVA procedure. Terms were added as long as the Wilk’s lambda test had  $P < 0.05$

Data set	Best approximating models	n
Hatchlings	<u>inctime</u> + sex + <u>inctime * sex</u> or meant + sex + <u>meant * sex</u>	125
Juveniles at 10 months	<u>inctime</u> + sex + <u>inctime * sex</u> or meant + sex + <u>meant * sex</u> or <u>inctime</u> + clutch + <u>inctime * clutch</u>	124

(b) Models that best represent each size trait respectively from general linear model procedure results ranked by Akaike information criteria (AIC<sub>c</sub>). All models within four of the lowest AIC<sub>c</sub> are listed. The most important term is underlined

Data set	Best approximating models	n
<i>Hatchlings</i>		
Jaw length	<u>cte</u> + sex + <u>cte * sex</u>	125
Jaw width	<u>vart</u>	
SVL	<u>cte</u> + sex + <u>cte * sex</u> or <u>inctime</u> + <u>clutch</u>	
<i>Total length</i>	<u>inctime</u> + <u>clutch</u>	124
Mass	<u>vart</u> + sex + <u>sex * vart</u>	
<i>Juveniles at 10 months</i>		
Jaw length	<u>vart</u> or <u>vart</u> + sex + <u>sex * vart</u>	
Jaw width	<u>meant</u>	
SVL	<u>vart</u>	
Total length	<u>cte</u>	
Mass	<u>vart</u>	

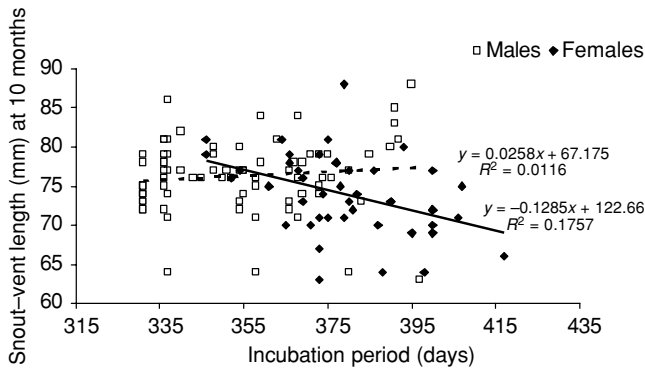
than females, but contrary to expectations of effects on females, increased temperature variance did not result in smaller (or larger) jaw lengths by 10 months (Fig. 4).

Variation in nest temperature was a significant influence on all size traits, indicated by inclusion of either CTE or nest temperature variance factors in models (Table 4b). However, CTE and nest temperature variance are the temperature variables that best predicted sex (Nelson *et al.*, in press), indicating temperature factors and sex were confounded as for artificial incubation treatments.

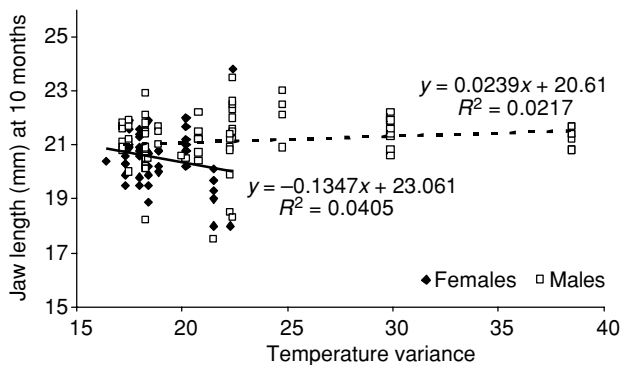
Parental influences on naturally incubated hatchlings were apparent, although not as obvious as for artificially incubated hatchlings. The availability of the less sensitive measure of clutch vs. initial egg mass and clutch for artificial incubation analyses probably explains this disparity. Clutch identity influenced total length and SVL of naturally incubated hatchlings.

**Natural vs. artificial incubation**

Hatchlings from artificial and natural incubation environments had similar length (SVL and total length) and mass. However, naturally incubated hatchlings had



**Fig. 3.** Sex-specific size vs. incubation period for naturally incubated tuatara *Sphenodon punctatus* juveniles. Females that incubated longer were smaller.



**Fig. 4.** The interaction between nest temperature variance and sex has a significant influence on jaw length. Female tuatara *Sphenodon punctatus* from more variable nests had smaller jaws.

smaller heads (measured by jaw length and width) than artificially incubated hatchlings (Table 1). By 10 months of age, naturally incubated juveniles were significantly smaller in all measurements than those from the constant artificial treatments. All naturally incubated juveniles had lost part of their tail (also affecting mass), compared to fewer incidences of tail-loss (73 %) and smaller amounts of tissue lost from tails of artificially incubated juveniles (Fig. 1).

## DISCUSSION

### Influences on size of tuatara

Parental effects predominantly influence size of hatchling tuatara, and they are still important at 10 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. Our method of ranking models penalized models that include factors, such as clutch, that have high degrees of freedom. Clutch size was small (mean = 7.6 eggs), 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 & Brooks, 1994; Congdon, Fischer & Gatten, 1995; Reichling & 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 covaried with incubation temperature variables as we wanted to disturb nests as little as possible to allow investigation of TSD in nature (Nelson *et al.*, in press).

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 vs. GLM/AIC<sub>c</sub>). 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 & Seymour, 1990; Van Damme *et al.*, 1992; Janzen, 1993), while others do not (e.g. Packard & Phillips, 1994; Reichling & Gutzke, 1996; Booth, 1998; Angilletta, Winters & Dunham, 2000). Results vary even between studies of the same species (Brooks *et al.*, 1991; Congdon *et al.*, 1999), because of variation in experimental designs, types of analyses and factors analysed. Interestingly there is no significant effect on size at hatching for naturally incubated tuatara, even though they have an extended incubation duration due to the onset of winter compared to embryos in artificial incubation conditions.

Incubation temperature significantly influences size by the time artificially incubated tuatara reach 10 months of age, despite similar rearing conditions post-hatching. Juveniles were exposed to similar amounts of food throughout the rearing period, regardless of size or hatching time, hence the level of competition within an enclosure would be expected to be similar across all enclosures for any particular age group. However, competition for food at 1 month of age in the social surroundings these juveniles experienced is likely to be less than at 10 months. The level of food available to juveniles throughout the study period was in excess of what they would be expected to experience in nature. 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).

At 10 months, juveniles that hatched from eggs incubated at 18 °C are smaller than those incubated at warmer temperatures. The 18 °C treatment also slightly reduces hatching success, suggesting this treatment may be nearing the lower limit for successful incubation of tuatara eggs. Similar effects from suboptimal incubation regimes occur in crocodiles (Webb & Smith, 1984), alligators (Joanen *et al.*, 1987) and geckos (Tousignant & Crews, 1995), although size differences are usually apparent at hatching in those species. Note, however, that juveniles from eggs incubated at 18 °C are still larger by 10 months of age than those incubated naturally.

### Adaptive significance of TSD for tuatara

Hatchling sex and parental effects confounded analysis of the influence of temperature on size of hatchlings incubated naturally. Also, water content of nests was not monitored. Nevertheless, some interesting patterns emerged in sizes of naturally incubated juveniles. Hatchlings with the longest SVLs developed in nests with a constant temperature equivalent between 21 and 23 °C. This corresponds well with the threshold temperature for sex determination in constant artificial incubation conditions (between 21 and 22 °C), and artificial incubation temperatures that produce the largest hatchlings by 10 months. Thermal variation during embryonic development has little effect on size in male hatchlings, but females that incubate for longer are 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, as predicted by theoretical models for the adaptive significance of TSD, are known in other species of reptiles (e.g. Shine *et al.*, 1997). The female phenotype is more sensitive than the male phenotype to thermal regime in *Bassiana duperreyi*, a scincid lizard where sex is affected by sex chromosomes and temperature (Elphick & Shine, 1999; Shine, Elphick & Donnellan, 2002). These patterns have been proposed as plausible explanations for the adaptive advantage of TSD (Charnov & Bull, 1977; Shine, Elphick & Harlow, 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 & Burghardt, 1974; Fox, 1978; McKnight & 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; but see Valenzuela & Janzen, 2001). Female tuatara aggressively compete for nest sites in discrete rookery 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 often guard their nest sites, albeit for short periods of time relative to the nesting season (authors, pers. obs.). 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, Mathies & Warner, 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 water, prey and refugia from that experienced by later hatchlings, and hence convey fitness advantages (Olsson *et al.*, 1996; Sinervo & Doughty, 1996). Juveniles that hatch earlier have a greater amount of time before winter to grow and find refugia. In tuatara, this pattern may be very important, as hatching occurs over at least 5 months (total incubation duration is between 11 and 16 months (McIntyre, 1997; authors, pers. obs). 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 & 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, Zappalorti & Gochfeld, 1987; O'Steen, 1998). Growth rates of turtles diverge 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.

Consequences of incubation environment for quality of young can last months, even years in other reptiles (Joanen *et al.*, 1987; Gutzke & Crews, 1988; Burger, 1989; Qualls & Shine, 1996; Roosenburg & Kelley, 1996; O'Steen, 1998). However, quality is subjective. Assessing offspring quality by measuring body size alone may be misleading (Ford & Seigel, 1994). Survival is not related to size in snapping turtles *Chelydra serpentina* (Congdon *et al.*, 1999). Furthermore, performance measures on snapping turtles are not necessarily intuitive. Less active turtle hatchlings are more likely to survive their first year, as their cryptic coloration protects them from visual predators (Janzen, 1995). Juvenile tuatara have cryptic coloration, but only limited work has been done on size-based performance; smaller tuatara tend to be faster (Pepperell, 1982). Smaller lizards (e.g. *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, Brown & DeMarco, 1982).

### Artificial vs. natural incubation

Artificial incubation conditions result in larger juveniles by 10 months of age than natural incubation conditions, despite similar sizes at hatching and similar rearing conditions. Jaw sizes of juveniles incubated in artificial conditions are larger at hatching than those from natural incubation conditions, potentially allowing greater access to food. However, naturally incubated juveniles are more aggressive, with extensive tail loss caused by attacks from



enclosure mates apparent from four months of age (N. J. Nelson, pers. obs.).

As generalities for size and performance data amongst reptiles do not apply, and there is limited information for tuatara, we can only speculate on the longer term consequences of size differences. For example, variation in size could affect reproductive capacity with consequences for lifetime reproductive success, especially as female tuatara have low annual reproductive output (approx. two 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, Watson & McFadden, 1994; Tyrrell, Cree & Towns, 2000). Either strategy could result in greater lifetime reproductive success (Roosenburg & Kelley, 1996).

The number and quality of offspring released into the wild determines the success of captive breeding and release programmes (Ford & Seigel, 1994). Performance experiments to test the significance of size in juvenile tuatara are needed. If size of tuatara measures quality, then young produced by artificial incubation 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 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. Future artificial incubation to produce females may be better conducted at 20–21 °C.

## SUMMARY

Egg mass affects size of tuatara at hatching, but incubation temperature influences size by 10 months of age. Constant artificial incubation conditions result in larger juveniles by 10 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, we propose increasing the incubation temperature for producing females from 18 °C to 20–21 °C. Sexual dimorphism is not apparent at 10 months of age, although some traits show signs of diverging, for example jaw size. Evidence from size patterns of tuatara incubated in natural nests supports differential fitness models for the adaptive significance of TSD.

## Acknowledgements

Special thanks to Alison Cree, Bryan Gartrell, Arthur Georges, Stephen Sarre, David Lambert, Geoff Birchard, Peter Gaze, Nga Manu Nature Reserve, and two anonymous referees. Research was supported by the Marsden Fund (Grant Number: 98-MAU-005) and

Victoria University of Wellington, and conducted 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, guardians of Takapourewa (Stephens Island), approved the research programme.

## REFERENCES

- Alberts, A. C., Perry, A. M., Lemm, J. M. & Phillips, J. A. (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. & Warner, D. A. (2000). Effect of incubation temperature on morphology, growth and survival of juvenile *Sceloporus undulatus*. *Herp. Monogr.* **14**: 420–431.
- Angilletta, M. J. Jr, Winters, R. S. & Dunham, A. E. (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*. London: HarperCollins Academic.
- Berry, J. F. & Shine, R. (1980). Sexual size dimorphism and sexual selection in turtles (order Testudines). *Oecologia* **44**: 185–191.
- Boby, M. L. & Brooks, R. J. (1994). Interclutch and interpopulation variation in the effects of incubation conditions on sex, survival and growth of hatchling snapping turtles (*Chelydra serpentina*). *J. Zool. (Lond.)* **233**: 233–257.
- Booth, D. T. (1998). Incubation of turtle eggs at different temperatures: do embryos compensate for temperature during development? *Physiol. Zool.* **71**: 23–26.
- Brooks, R. J., Bobyn, M. L., Galbraith, D. A., Layfield, J. A. & Nancekivell, E. G. (1991). Maternal and environmental influences on growth and survival of embryonic and hatchling snapping turtles (*Chelydra serpentina*). *Can. J. Zool.* **69**: 2667–2676.
- Bull, J. J. & Vogt, R. C. (1981). Temperature sensitive periods of sex determination in emydid turtles. *J. Exp. Zool.* **218**: 435–440.
- Burger, J. (1989). Incubation temperature has long-term effects on behaviour of young pine snakes (*Pituophis melanoleucus*). *Behav. Ecol. Sociobiol.* **24**: 201–207.
- Burger, J. (1990). Effects of incubation temperature on behaviour of young black racers (*Coluber constrictor*) and kingsnakes (*Lampropeltis getulus*). *J. Herpetol.* **24**: 158–163.
- Burger, J., Zappalorti, R. T. & Gochfeld, M. (1987). Effects of incubation temperature on hatchling pine snakes *Pituophis melanoleucus*. *Comp. Biochem. Physiol.* **87A**: 727–732.
- Burnham, K. P. & Anderson, D. R. (1998). *Model selection and inference. A practical information-theoretic approach*. USA: Springer-Verlag NY Inc.
- Cagle, K. D., Packard, G. C., Miller, K. & Packard, M. J. (1993). Effects of the microclimate in natural nests on development of embryonic painted turtles, *Chrysemys picta*. *Funct. Ecol.* **7**: 653–660.
- Cartland, L. K. & Grimmond, N. M. (1994). The effect of temperature on the metabolism of juvenile tuatara, *Sphenodon punctatus*. *N. Z. J. Zool.* **21**: 373–378.
- Charnov, E. L. & Bull, J. J. (1977). When is sex environmentally determined? *Nature* **266**: 828–830.
- Congdon, J. D., Fischer, R. U. & Gatten, R. E. 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., Kinney, O. M. & Yeomans, S. R. (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. *N. Z. J. Zool.* **21**: 351–372.
- Cree, A. & Butler, D. (1993). *Tuatara recovery plan (Sphenodon spp.)*. Threatened Species Recovery Plan Series No. 9. Wellington: Department of Conservation, New Zealand.
- Cree, A., Cockrem, J. F., Brown, M. A., Watson, P. R., Guillette, L. J. Jr., Newman, D. G. & Chambers, G. K. (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. & Daugherty, C. H. (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. In *New Zealand herpetology*: 237–250. Newman, D. G. (Ed.). New Zealand Wildlife Service Occasional Publication No. 2. Wellington: Department of Internal Affairs.
- Deeming, D. C. & Ferguson, M. W. J. (1991). Physiological effects of incubation temperature on embryonic development in reptiles and birds. In *Egg incubation: its effects on embryonic development in birds and reptiles*: 147–171. Deeming, D. C. & Ferguson, M. W. J. (Eds). Cambridge: Cambridge University Press.
- Dendy, A. (1899). Outlines of the development of the tuatara, *Sphenodon (Hatteria) punctatus*. *Quart. J. Microscop. Soc.* **42**: 1–87.
- Elphick, M. J. & Shine, R. (1998). Longterm effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards (*Bassiana duperreyi*, *Scincidae*). *Biol. J. Linn. Soc.* **63**: 429–447.
- Elphick, M. J. & Shine, R. (1999). Sex differences in optimal incubation temperature in a scincid lizard species. *Oecologia* **118**: 431–437.
- Ferguson, G. W. & Fox, S. F. (1984). Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. *Evolution* **38**: 342–349.
- Ferguson, G. W., Brown, K. L. & DeMarco, V. G. (1982). Selective basis for the evolution of variable egg and hatchling size in some iguanid lizards. *Herpetologica* **38**: 178–188.
- Ford, N. B. & Seigel, R. A. (1994). Phenotypic plasticity: implications for captive breeding and conservation programs. In *Captive management and conservation of amphibians and reptiles. Contributions to herpetology*, **11**: 175–182. Murphy, J. B., Adler, K. & Collins, J. T. (Eds). New York: Society for the Study of Amphibians and Reptiles.
- Fox, S. F. (1978). Natural selection on behavioural phenotypes of the lizard *Uta stansburiana*. *Ecology* **59**: 834–847.
- Froese, A. D. & Burghardt, G. M. (1974). Food competition in captive juvenile snapping turtles, *Chelydra serpentina*. *Anim. Behav.* **22**: 735–740.
- 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. & Stoutjesdijk, R. (1994). Hatchling sex in the marine turtle *Caretta caretta* is determined by proportion of development at a temperature, not daily duration of exposure. *J. Exp. Zool.* **270**: 432–444.
- Gutzke, W. H. N. & Crews, D. (1988). Embryonic temperature determines adult sexuality in a reptile. *Nature* **332**: 832–834.
- Gutzke, W. H. N. & Packard, G. C. (1987). Influence of hydric and thermal environments on eggs and hatchlings of bull snakes *Pituophis melanoleucus*. *Physiol. Zool.* **60**: 9–17.
- Head, G., May, R. M. & Pendleton, L. (1987). Environmental determination of sex in reptiles. *Nature* **329**: 198–199.
- Huey, R. B. (1982). Temperature, physiology and the ecology of reptiles. In *Biology of the reptilia*: 25–91. Gans, C. & Pough, F. H. (Eds). London: Academic Press.
- IUCN (1996). *Red list of threatened animals*. Gland, Switzerland: IUCN.
- Janzen, F. J. (1993). The influences of incubation temperature and family on eggs, embryos, and hatchlings of the smooth softshell turtle (*Apalone mutica*). *Physiol. Zool.* **66**: 349–373.
- Janzen, F. J. (1995). Experimental evidence for the evolutionary significance of temperature-dependent sex determination. *Evolution* **49**: 864–873.
- Joanen, T., McNease, L. & Ferguson, M. W. J. (1987). The effects of egg incubation temperature on post-hatching growth of American alligators. In *Wildlife management: crocodiles and alligators*: 533–537. Webb, G. J. W., Manolis, S. C. & Whitehead, P. J. (Eds). Sydney: Surrey Beatty.
- Lang, J. W., Andrews, H. & Whitaker, R. (1989). Sex determination and sex ratios in *Crocodylus palustris*. *Am. Zool.* **29**: 935–952.
- Lawrence, M. H. (1997). *The importance of lizards to seed dispersal of native montane fleshy fruits*. Unpublished MSc thesis, Canterbury, New Zealand.
- Lee, C. & Rawlings, J. O. (1982). Design of experiments in growth chambers – uniformity trials in North Carolina State University phytotron. *Crop Science* **22**: 551–558.
- McIntyre, M. (1997). *Conservation of the tuatara*. Wellington: Victoria University Press.
- McKnight, C. M. & Gutzke, W. H. N. (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. & Packard, M. J. (1987). Hydric conditions during incubation influence locomotor performance of hatchling snapping turtles. *J. Exp. Biol.* **127**: 401–412.
- Nelson, N. J. (1998). *Conservation of Brothers Island tuatara, Sphenodon guntheri*. Unpublished MConSc thesis, Victoria University of Wellington, New Zealand.
- Nelson, N. J., Cree, A., Thompson, M. B., Keall, S. N. & Daugherty, C. H. (in press) Temperature-dependent sex determination in tuatara. In *Temperature dependent sex determination in vertebrates*. Valenzuela, N. & Lance, V. (Eds). Washington DC: Smithsonian Books.
- Newman, D. G., Watson, P. R. & McFadden, I. (1994). Egg production by tuatara on Lady Alice and Stephens Islands. *N. Z. J. Zool.* **21**: 387–398.
- Olsson, M., Gullberg, A., Shine, R., Madsen, T. & Tegelstrom, H. (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*. *J. Exp. Biol.* **201**: 439–449.
- Packard, G. C. & Packard, M. J. (1988). The physiological ecology of reptilian eggs and embryos. In *Biology of the reptilia*, **16**, *Ecology B*: 523–603. Gans, C. & Huey, R. B. (Eds). London: Academic Press.
- Packard, G. C. & Phillips, J. A. (1994). The importance of the physical environment for the incubation of reptilian eggs. In *Captive management and conservation of amphibians and reptiles*, **11**: 195–208. Murphy, J. B., Adler, K. & Collins, J. T. (Eds). New York: Society for the Study of Amphibians and Reptiles.
- Pepperell, J. G. (1982). Tuatara *Sphenodon punctatus* locomotion: a summary. In *New Zealand herpetology*: 207–211. Newman, D. G. (Ed.). Wildlife Service Occasional Publication No. 2. Wellington: Department of Internal Affairs.
- Phillips, J. A., Garel, A., Packard, G. C. & Packard, M. J. (1990). Influence of moisture and temperature on eggs and embryos of green iguanas (*Iguana iguana*). *Herpetologica* **46**: 238–245.

- Qualls, C. P. & Shine, R. (1996). Reconstructing ancestral reaction norms: an example using the evolution of reptilian viviparity. *Funct. Ecol.* **10**: 688–697.
- Reichling, S. B. & Gutzke, W. H. N. (1996). Phenotypic consequences of incubation environment in the African elapid genus *Aspidelaps*. *Zoo Biol.* **15**: 301–308.
- Reinhold, K. (1998). Nest-site philopatry and selection for environmental sex determination. *Ecol. Evol.* **12**: 245–250.
- Rhen, T. & Lang, J. W. (1995). Phenotypic plasticity for growth in the common snapping turtle: effects of incubation temperature, clutch, and their interaction. *Am. Nat.* **146**: 726–747.
- Roosenburg, W. M. & Kelley, K. C. (1996). The effect of egg size and incubation temperature on growth in the turtle, *Malaclemys terrapin*. *J. Herpetol.* **30**: 198–204.
- Schwarzkopf, L. & Brooks, R. J. (1987). Nest-site selection and offspring sex ratio in painted turtles, *Chrysemys picta*. *Copeia* **1987**: 53–61.
- Shine, R. (1999). Why is sex determined by nest temperature in many reptiles? *TREE* **14**: 186–189.
- Shine, R., Elphick, M. J. & Donnellan, S. (2002). Co-occurrence of multiple, supposedly incompatible modes of sex determination in a lizard population. *Ecol. Lett.* **5**: 486–489.
- Shine, R., Elphick, M. J. & Harlow, P. S. (1995). Sisters like it hot. *Nature* **378**: 451–452.
- Shine, R., Elphick, M. J. & Harlow, P. S. (1997). The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology* **78**: 2559–2568.
- Shine, R. & Harlow, P. S. (1996). Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology* **77**: 1808–1817.
- Sinervo, B. & Doughty, P. (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. & Kemp, S. J. (1994). Effects of incubation conditions on sex determination, hatching success, and growth of hatchling desert tortoises, *Gopherus agassizii*. *Herp. Monogr.* **8**: 103–116.
- Steyermark, A. C. & Spotila, J. R. (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. & Coe, M. J. (1979). The natural regulation of giant tortoise populations on Aldabra Atoll: recruitment. *Philos. Trans. R. Soc. Lond. B* **286**: 177–188.
- Thompson, M. B. (1990). Incubation of eggs of tuatara, *Sphenodon punctatus*. *J. Zool. (Lond.)* **222**: 303–318.
- Thompson, M. B., Newman, D. G. & Watson, P. R. (1991). Use of oxytocin in obtaining eggs from tuatara (*Sphenodon punctatus*). *J. Herpetol.* **25**: 101–104.
- Thompson, M. B., Packard, G. C., Packard, M. J. & Rose, B. (1996). Analysis of the nest environment of tuatara *Sphenodon punctatus*. *J. Zool. (Lond.)* **238**: 239–251.
- Tousignant, A. & Crews, D. (1995). Incubation temperature and gonadal sex affect growth and physiology in the leopard gecko (*Eublepharis macularis*), a lizard with temperature-dependent sex determination. *J. Morphol.* **224**: 159–170.
- Tyrrell, C. L., Cree, A. & Towns, D. R. (2000). *Variation in reproduction and condition of northern tuatara (Sphenodon punctatus punctatus) in the presence and absence of kiore*. Science for Conservation No. 153. Wellington: Department of Conservation, New Zealand.
- Valenzuela, N. & Janzen, F. J. (2001). Nest-site philopatry and the evolution of temperature-dependent sex determination. *Evol. Ecol. Res.* **3**: 779–794.
- Van Damme, R., Bauwens, D., Brana, F. & Verheyen, R. F. (1992). Incubation temperature differentially affects hatching time, egg survival and sprint speed in the lizard *Podarcis muralis*. *Herpetologica* **48**: 220–228.
- Webb, G. J. W., Beal, A. M., Manolis, S. C. & Dempsey, K. E. (1987). The effects of incubation temperatures on sex determination and embryonic development rate in *Crocodylus johnstoni* and *C. porosus*. In *Wildlife management: crocodiles and alligators: 507–531*. Webb, G. J. W., Manolis, S. C. & Whitehead, P. J. (Eds). Sydney: Surrey Beatty.
- Webb, G. J. W. & Smith, A. M. A. (1984). Sex ratio and survivorship in the Australian freshwater crocodile *Crocodylus johnstoni*. *Symp. Zool. Soc. Lond.* **52**: 319–355.
- Whitehead, P. & Seymour, R. S. (1990). Patterns of metabolic rate in embryonic crocodylians *Crocodylus johnstoni* and *Crocodylus porosus*. *Physiol. Zool.* **63**: 334–352.
- Yntema, C. L. (1979). Temperature levels and period of sex determination during incubation of eggs of *Chelydra serpentina*. *J. Morphol.* **159**: 17–28.