Do TSD, sex ratios, and nest characteristics influence the vulnerability of tuatara to global warming?

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\textbf{Abstract.} Tuatara (\textit{Sphenodon punctatus}) are threatened New Zealand reptiles with temperature-dependent sex determination (TSD). Higher incubation temperatures produce males, and less than 1 °C separates production of males and females. We investigated variability in nesting ecology to assess whether global warming is likely to result in increasingly male-biased populations. We examined nesting seasons during 1998/1999 and 2002/2003 in New Zealand’s largest tuatara population on Stephens Island, and collected hourly temperature recordings and physical descriptions from 70 nests. Nest depths were not significantly different between years, and ranged from 10 to 230 mm from the soil surface to the top egg. Incubation temperatures in successful nests throughout the year-long incubation period ranged from 1.6 to 38.4 °C. Sex ratios of nests were correlated with incubation temperature: 64% males were produced in 1998/1999, a relatively warm season, but we predict an equal sex ratio was produced in 2002/2003. Although temperatures varied over the 2002/2003 season with respect to monthly long-term averages, 2002 was the second warmest year on record. Stephens Island supports a wide range of nesting habitat, a relatively large population of tuatara, and nest characteristics are highly variable. As such, this population is likely to be resilient to global warming in the short term because an equal sex ratio was predicted from a relatively warm season. However, most other islands where tuatara occur are smaller, have smaller populations, and have fewer open areas for nesting and/or shallower soils. These conditions are more likely to produce a male bias in hatchlings because female
tuatara do not appear to vary construction of nests with respect to temperature or location. In the extreme, this could lead to the extinction of small populations. © 2004 Elsevier B.V. All rights reserved.

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1. Introduction

Reptiles with temperature-dependent sex determination (TSD), where embryonic sex is determined by incubation temperatures, have survived extreme climate change before [1]. However, the short time frame and the scale of global temperature increase predicted (+1.4–5.8 °C in the next 100 years)[2] may result in sex ratio imbalances that will threaten population persistence. Species with narrow transitional ranges (the range of incubation temperatures that produce 100% of one sex to 100% of the other sex) and long generation times (indicating limited potential to respond to rapid changes in climate) have four alternatives: to modify their geographic range, to convert to genetic sex determination, to go extinct, or to modify their nesting behaviour [3,4]. Biased sex ratios are already known for reptiles with TSD [5]. A general warming trend is already evident in sea turtle nests (Chelonia mydas at Ascension Island) over the past 100 years [6]. In addition, findings so far point to nest site selection by female reptiles for hatching success rather than sex ratio manipulation (e.g. Refs. [4,7,8]).

Tuatara (Sphenodon), long-lived sole surviving members of the reptilian Order Sphenodontia, are restricted to small off-shore islands of New Zealand [9]. The two species (S. guntheri and S. punctatus) both have the rare Type 1b pattern of TSD, where males are produced from nests with higher incubation temperatures [10,11]. There is no evidence so far to suggest they exhibit the type II pattern of TSD where females are produced at both extremes and males at intermediate incubation temperatures [11]. The pivotal temperature of the largest population of tuatara (21.7 °C, S. punctatus on Stephens Island) is relatively low for reptiles [12]. In addition, sex determination of embryos from this population occurs over a narrow transitional range of less than 1 °C [11]. The adult sex ratio on Stephens Island is estimated to be 50% males [13]. The only extant population of S. guntheri on North Brother Island comprises 60% adult males [14].

Nesting occurs in spring from early November, and female tuatara lay a clutch of eggs only about every 2–5 years [15,16]. Shallow subterranean nests are constructed in open areas, not in forest habitat where temperatures are too low for successful development of embryos. On Stephens Island, nesting occurs in paddocks grazed by sheep, open cliff faces and rocky outcrops, and paths [16].

We investigate whether the tuatara’s rare pattern of TSD, biased sex ratios, and diversity of nest characteristics and habitat influence the likelihood of male-biased populations that potentially threaten population persistence as a result of global warming. As the first step towards an inventory we ask the following questions: (1) What is the hatchling sex ratio? (2) Are there between-year variations in hatchling sex ratio? (3) Are there between-year variations in nest construction or time of laying?
2. Methods

Nests of tuatara were investigated during the annual nesting seasons in 1998 (3 weeks, November 1–21)[11] and 2002 (6 weeks, October 28–December 7) on Stephens Island (150 ha), New Zealand. Temperature data loggers (waterproof Stowaway® TidbiT®, dimensions: 30×41×17 mm, Onset Computer, MA, USA) set to record hourly measurements were inserted into nests spread throughout rookeries in sheep paddocks and on rocky outcrops (25 nests in 1998 and 45 nests in 2002). Back-fill of nests was excavated, and the data logger was placed beside the eggs. Nests were carefully re-filled and left intact until the following nesting season (October 1999 (11 months incubation) and December 2003 (13 months incubation), respectively). Physical descriptions were recorded for each nest, including depth to the top layer of eggs and location/rookery (for example, winch house paddock, house #3 paddock). The number of eggs and hatching success for each nest were recorded upon excavation of nests to retrieve data loggers. All hatchlings and/or eggs were collected from nests monitored during the 1998/1999 season and taken to Victoria University of Wellington to complete incubation at 22 °C (sex had already been determined in nests). Hatchlings were reared in captivity and sexed by laparoscopy at approximately one year of age [11]. Temperature recordings during the middle period of development were used to estimate the sex ratio of hatchlings from each nest in 2002/2003. The period of sex determination for tuatara is not known, but the middle third of development is when sex determination occurs in other reptiles [17].

Temperature records were summarised by calculating mean, minimum and maximum throughout incubation, and the constant temperature equivalent (CTE) during the middle period of development for each nest. The CTE is defined as the temperature above which half of embryonic development occurs [18,19]. In calculating the CTE, the models take account of the variance in temperatures, providing a more meaningful representation of the nest environment than the mean temperature. The inputs used for the Georges’ model were developmental zero of 11.1 °C, reference temperature 18 °C with incubation period of 264 days, pivotal temperature 21 °C, and hourly temperature records for each nest [11]. Outputs from the model include CTE and developmental progress (expressed as a proportion) for each 24-h period. Mean CTE during 0.45–0.55 of development was estimated to be within the period of sex determination for tuatara. Sex ratios predicted using CTE during 0.45–0.55 of development matched closely with known sex ratios of hatchlings from nests in 1998/1999. 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, and females are predicted to be produced if the CTE is lower than the threshold. Previously, CTEs during February had been used due to the good correlation with known sex ratios in the 1998/1999 nesting season [11]. However, we did not want to make the assumption that 0.45–0.55 of development falls during February in all years. A wider period, for example the middle third of development (0.33–0.66), would have resulted in inclusion of temperature data covering several months (and in some cases over winter), potentially introducing biases into sex ratio estimates for cooler nests.

Analyses of variance were performed using SAS on the following: nest depth as the dependent variable with nesting season (year), date of laying and rookery location as
predictors; temperature of nest including minimum, maximum, mean or CTE as dependent variables with nest depth and year as predictors; and hatching success as the dependent variable and nest depth as the predictor.

Akaike’s information criteria (AIC) [20,21] were used to compare logistic and asymmetric logistic models [22] for the proportion of male tuatara produced from nests in 1998/1999 with respect to nest CTE (mean during 0.45–0.55 of development) in R [23]. Proportions of males produced from nests in 2002/2003 were predicted using these models with input values of mean CTE during 0.45–0.55 of development for each nest in 2002/2003 and clutch size. Unless otherwise stated, all values presented are means±1 SE.

3. Results

Mean depth of nests was 103.5±9.2 mm (range 40–200 mm) in 1998/1999, and was not significantly different from 111.0±6.3 mm (range 10–230 mm) in 2002/2003 (Table 1; \(F_{(1,60)}=0.55; P=0.459\)). Nest depths were not significantly correlated with the date of laying or rookery location (\(F_{(1,60)}=0.36; P=0.551\) and \(F_{(8,53)}=1.10; P=0.377\)). Depth significantly influenced variability of nest temperatures (minimum: \(F_{(1,60)}=28.45; P<0.001\); maximum: \(F_{(1,60)}=17.25; P<0.001\); CTE: \(F_{(1,60)}=5.50; P=0.022\)) but not mean temperatures (\(F_{(1,60)}=0.16; P=0.689\)). Nest depth influenced hatching success (\(F_{(2,40)}=2.35; P<0.01\)). For example, nests shallower than 50 mm were less than 50% successful.

Mean minimum and maximum temperatures in November 1998 during the laying season (measured at the meteorological station on Stephens Island; 10.3 and 14.9 °C, respectively) were higher than over the same period in 2002 (9.6 and 14.4 °C, respectively). Mean temperature throughout the lengthy incubation period in nests was higher in 1998/1999 (16.5±0.1 °C) than 2002/2003 (15.8±0.1 °C; \(F_{(1,60)}=24.93; P<0.001\)), but the range of temperatures experienced by successful nests was wider in the second season (2.9–34.4 °C in 1998/1999; 1.6–38.4 °C in 2002/2003). Minimum temperatures (but not maximum or CTE) were lower in 2002/2003 (\(F_{(1,60)}=9.38;\)

### Table 1
Characteristics and temperatures of tuatara nests over two seasons: 1998/1999 (25 nests) and 2002/2003 (45 nests)

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>Depth (mm)</th>
<th>No. of eggs</th>
<th>Hatching success (%)</th>
<th>Temperature (°C)</th>
<th>CTE 0.45–0.55 Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Min.</td>
</tr>
<tr>
<td>1998/1999</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>103.5</td>
<td>9</td>
<td>66</td>
<td>16.5</td>
<td>7.1</td>
</tr>
<tr>
<td>Min.</td>
<td>40</td>
<td>3</td>
<td>0</td>
<td>15.3</td>
<td>2.9</td>
</tr>
<tr>
<td>Max.</td>
<td>200</td>
<td>13</td>
<td>100</td>
<td>17.6</td>
<td>8.7</td>
</tr>
<tr>
<td>SE</td>
<td>9.2</td>
<td>0.1</td>
<td>8</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>2002/2003</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>110.9</td>
<td>8.8</td>
<td>55</td>
<td>15.8</td>
<td>5.6</td>
</tr>
<tr>
<td>Min.</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>14.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Max.</td>
<td>230</td>
<td>22a</td>
<td>100</td>
<td>17.0</td>
<td>10.4</td>
</tr>
<tr>
<td>SE</td>
<td>6.3</td>
<td>0.6</td>
<td>6</td>
<td>0.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* May include more than one clutch. CTE refers to constant temperature equivalent of nests.
Mean temperatures of nests were significantly different across rookeries \( (F_{(8,53)}=3.07; P=0.006) \).

Mean clutch size did not differ between years: \( 9\pm0.6 \) eggs in 1998/1999 and \( 8.8\pm0.6 \) eggs in 2002/2003. Hatching success was significantly higher in the first season (66% compared to 55%; \( X^2_{1}=7.271, P=0.007 \)).

Nests in 1998/1999 produced 64% males. The asymmetric logistic model (AIC 124.9; # parameters=3) was not a significantly better predictor of the pattern of sex determination than the logistic model (AIC 124.2; # parameters=2; \( X^2_{1}=1.275 \)), so the logistic model (pivotal temperature=21.3°C; steepness parameter=0.97) was used to predict the sex ratio of hatchlings from the 2002/2003 season nests (Fig. 1). We predict an equal sex ratio of hatchlings was produced from nests incubated during the 2002/2003 season, comprising 111.3±7.1 males and 105.7±7.1 females. Sex ratios and numbers of nests varied across the rookeries, with only the winch house and house #3 paddocks having reasonable numbers of nests in both seasons. The winch house paddock produced a preponderance of male-biased nests from both seasons, while the house #3 paddock produced an equal sex ratio in 1998/1999 and a female-biased sex ratio from 2002/2003. No rookeries produced female-biased sex ratios in 1998/1999.

**4. Discussion**

We found a male-biased sex ratio in our first season of monitoring tuatara nests, but estimated an equal sex ratio from the second season. Several differences exist between the two seasons. Although we encompassed the preponderance of nesting in each instance, nests in the first season in 1998/1999 were marked over 3 weeks compared to 6 weeks in the second season (2002/2003). Temperatures during November, the primary period for
laying, were higher in the first season. Warmer weather during nest construction may have resulted in the shortened nesting season. Deposition of eggs earlier in the season may result in higher average temperatures of nests due to nest exposure to a longer summer period. Mean incubation temperature of nests was higher in the first season. However, we do not know whether individual female tuatara that lay eggs only every 2–5 years \[15\] were the same (or different) between the two seasons, and whether individuals characteristically lay at a certain time during a season (for example early or late). Another difference between seasons was the number of nests. Almost twice as many nests were monitored in the second season. However, nests in the first season were a sample selected to represent the diversity of nest site characteristics observed during that season. Not all nests were monitored in 1998/1999 as during that study eggs were also collected for artificial incubation \[11,24\]. Lastly, the sex ratio in the first season was measured directly by laparoscopy of hatchlings, compared to estimation of sex ratios from the second season using TSD models developed using the first season’s data. Direct measurements of sex ratio cannot be conducted on all individuals in all seasons due to logistics and restrictions involved in collecting hatchlings of a protected species and holding them for a year to enable laparoscopies to be conducted. We will continue to refine our estimates of the sex determining period for tuatara and techniques for modelling temperatures of natural nests to estimate sex ratios.

Nest depths were not significantly different between seasons, among rookeries, or with respect to date of egg deposition, suggesting female tuatara on average do not construct nests according to environmental cues during nesting (e.g. relative temperature or day length) or nest site location (e.g. aspect or soil structure). However, the location of nests (rookery) affected temperatures, probably as a result of thermal properties of soil and aspects of rookeries. Variability of temperatures and hatching success in a nest were correlated with nest depth, suggesting that individual females have the potential to manipulate success and sex ratio of hatchlings depending on which rookery they choose and how they construct nests. Other species of reptiles demonstrate both species-specific and female-specific differences in nest site selection, to maximise embryonic survival and potentially to manipulate sex ratio (e.g. Ref. \[7\]). We do not know whether female tuatara construct similar nests in similar locations every time they nest. However, we have observed nesting in the same rookeries and the same areas within rookeries since 1986. Many of the nesting females have individual marks (toe-clips \[25\] or passive integrated transponders) that will allow us to investigate whether variability of sex ratios among years is a consequence of year to year variation in temperatures during egg deposition and/or incubation season, and hence likely to be influenced by global warming, or of nest construction and site selection of individuals depositing eggs in any particular year.

Temperatures on Stephens Island throughout the 1998/1999 summer were warmer than average based on records covering the past 35 years, including the warmest February and March on record in 1999. The average temperature in 1998 was 0.47 \(^{\circ}\)C warmer than usual in the Southern Hemisphere and the highest on record (NIWA National Climate Database). In contrast, temperatures on Stephens Island throughout the 2002/2003 summer were cooler than average in November, January and February, and warmer than average in December and March, although 2002 in the Southern Hemisphere was the second warmest year on record (0.36 \(^{\circ}\)C warmer than usual; NIWA National Climate Database). Warmer
periods during egg deposition are likely to result in greater nesting activity, but whether this translates into completion of nesting in a shorter period of time is uncertain. As female tuatara nest only every 2–5 years, we intend to investigate individual variation in nesting over time and patterns of nest variation over years where independence of females can be guaranteed.

Stephens Island supports a wide range of nesting habitat for tuatara, from artificially created areas like sheep paddocks to natural cliff edges. The variety and abundance of nesting habitat is proposed to be the reason this island supports the largest population of tuatara. Investigating how global warming may affect sex ratios of tuatara populations on Stephens Island is possible because nesting can be observed and relatively large amounts of data obtained. Results from two nesting seasons suggest sex ratios of tuatara populations will be resilient to global warming in the short term because an equal sex ratio was predicted from a relatively warm season. However, most other islands where tuatara occur are smaller, have fewer open areas for nesting and/or shallower soils. Additionally, nesting is comparatively difficult to monitor. As nest depth influences hatching success and sex ratio through temperature, and female tuatara do not appear to dig deeper nests in warmer years, a male bias in hatchlings may be more likely from tuatara nests on other islands. In the extreme, this could lead to extinction of small populations.

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References
