Behavioral variation in nesting phenology may offset sex-ratio bias in tuatara

Nicola J. Nelson1 | Susan N. Keall1 | Jeanine M. Refsnider2 | Anna L. Carter3

1School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand
2Department of Environmental Sciences, University of Toledo, Toledo, Ohio
3Department of Ecology, Evolution & Organismal Biology, Iowa State University, Ames, Indiana

Correspondence
Nicola Nelson, School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand.
Email: Nicola.Nelson@vuw.ac.nz

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Abstract
The nest environment for eggs of reptiles has lifelong implications for offspring performance and success, and, ultimately, for population viability and species distributions. However, understanding the various abiotic and biotic drivers of nesting is complex, particularly regarding variation in nesting behavior of females and consequences for sex ratios in species with temperature-dependent sex determination (TSD). We investigated how nest construction and nesting phenology affect the incubation environment of a reptile with TSD, the tuatara (Sphenodon punctatus), a species that is at risk from climate-mediated male bias in population sex ratios. Using longitudinal behavioral data, we addressed the following questions. (1) Does nesting behavior vary with seasonal or location cues? (2) Does variation in nesting phenology or nest construction affect the incubation environment? We aimed to investigate whether female tuatara could modify nesting behavior to respond to novel environments, including a warming climate, allowing for successful incubation and balanced population sex ratios, maintaining population viability throughout their historic range. We predicted that earlier nesting after warm winters increased the likelihood that females will be produced, despite the sex determining system where males are produced from warmer temperatures. Further research is needed to understand the extent to which nesting behavior varies by individual through time, and across the range of tuatara, and the importance of habitat variability in maintaining production of females under future climate warming.

KEYWORDS
incubation environment, nest site choice, nesting phenology, tuatara (Sphenodon punctatus)

1 INTRODUCTION

The abiotic environment influences community compositions, species distributions, population dynamics, and individual fitness. Organisms may be affected differently by their environments throughout ontogeny. For oviparous species, the nest environment has lifelong implications for offspring performance, survival, fitness, and, ultimately, for population viability and species distributions (McGaugh, Schwanz, Bowden, Gonzalez, & Janzen, 2010; Valenzuela & Lance, 2004; Warner & Shine, 2008a). However, the various abiotic and biotic drivers of nesting are complex (Warner & Shine, 2008a). Physical factors that influence the nest environment (e.g., temperature and water availability) vary depending on climate patterns, the timing of nesting and nest characteristics, such as location and shading (e.g., Ewert, Lang, & Nelson, 2005; Janzen & Morjan, 2001). Temperatures in natural nests have wide-ranging effects on hatching success and hatching size, growth, and morphology and can fluctuate from below developmental or lethal minimums to above lethal maximums throughout incubation (Hare, Pledger, & Daugherty, 2008; Qualls & Andrews, 1999; Shine, Elphick, & Barrott, 2003). Variability in behavior of nesting females adds a further layer of complexity, reflected in plasticity in how, when, and where females deposit their eggs. Nesting behavior among females may vary within and among populations and with respect to cues, including temperature, shading, and evidence left by conspecifics (e.g., Refsnider & Janzen, 2012; Refsnider, Daugherty, Keall, & Nelson, 2010; Telemeco, Elphick, & Shine, 2009).

For reptiles with temperature-dependent sex determination (TSD), the nest environment determines the sex of hatchlings, which differentiate into males or females according to a species' particular pattern of sex determination and the temperatures experienced during incubation, which affect both the rate of development and sex (reviewed by Valenzuela & Lance, 2004). The relationship between temperature and development is nonlinear, complicating understanding of how abiotic and biotic drivers interact, and affecting predictive power when contemplating implications of climate warming for the nesting
environment (e.g., Mitchell, Kearney, Nelson, & Porter, 2008; Pen et al., 2010; Telemeco, Abbott, & Janzen, 2013).

Species with TSD are proposed to be at risk due to climate warming, particularly those with low heritable genetic variation, long life histories, and/or the TSD pattern in which males are produced from warmer temperatures, which is likely to result in male-biased sex ratios thought to be suboptimal for population viability (Grayson et al., 2014; Hulin, Delmas, Girondot, Godfrey, & Guillon, 2009; Mitchell et al., 2008). However, phylogenetic relationships of reptiles and their patterns of sex determination support the theory that species with TSD have survived periods of extremely rapid warming in the past (Janzan & Phillips, 2006; Mitchell & Janzen, 2010; Rest et al., 2003; Steffensen et al., 2008), suggesting that reptiles with TSD nonetheless have some capacity to respond to rapid environmental change and thereby prevent substantial skews in sex ratios.

Evidence of the capacity of reptiles to respond to different environmental conditions arises from latitudinal and longitudinal studies. For example, among-family variation in sex ratio was detected in alligators (Alligator mississippiensis) and common snapping turtles (Chelydra serpentina) (Rhen & Lang, 1998), and the range of temperatures over which males are produced is expanded at higher latitudes in snapping turtles (Ewert et al., 2005). Three-lined skinks (Bassiana duperreyi) nest earlier and dig deeper nests in response to warming temperatures (Telemeco et al., 2009). Nest choice involving selection of shade is apparent in painted turtles (Eretmochelys imbricata) (Reifsneider et al., 2009, 2010). Nesting females were individually marked using passive integrated transponders (PIT tags; Allflex, ISO FDX-B; Reifsneider et al., 2009). Progress of nest construction was monitored nightly by recording accumulated burrow length (±10 mm) for each nesting attempt. Construction was evident from freshly deposited soil outside nest entrances, change in the length of the burrow, or the presence of a female tuatara digging.

We investigated how nest construction and nesting phenology affect the incubation environment of a reptile with TSD, the tuatara (Sphenodon punctatus), a species that is at risk from climate-mediated male bias in population sex ratios. Sex ratios are not well known for tuatara populations, but in one population, a significant (60–80%) male-biased adult sex ratio is already apparent (North Brother Island; Grayson et al., 2014; Mitchell et al., 2008). However, on nearby Stephens Island (Takapourewa), the population sex ratio is estimated currently at 1:1 (Moore, Daugherty, & Nelson, 2009). Female tuatara on Stephens Island nest every 2–4 years during austral spring (Cree, Thompson, Guilleltte, Hay, & McIntyre, 1989; Thompson, Packard, Packard, & Rose, 1996). Eggs incubate for 11–16 months, and only about 48% of eggs successfully incubate to hatching in the wild, likely due to nest disturbance from other females and temperature-mediated effects on water availability (Reifsneider, Keall, Daugherty, & Nelson, 2009; Thompson et al., 1996). Eggs incubating in shallow nests (<50 mm below soil surface) experience higher mortality (>50%; Nelson, Thompson, Pledger, Keall, & Daugherty, 2004). Incubation experiments using eggs from Stephens Island indicate sex determination in tuatara occurs at the beginning of the middle third of incubation (30% development; Nelson, Moore, Pillai, & Keall, 2010), with males produced from warm nests (Cree, Thompson, & Daugherty, 1995; Grayson et al., 2014; Mitchell et al., 2006). However, little is known about the influences on, or variation in, female behaviors associated with nesting that could affect population sex ratios. For example, in Cook Strait, nesting occurs in unforested, colonial rookeries; nesting under forest cover is not currently successful due to low temperatures (Cree et al., 1989; Thompson et al., 1996). Tuatara display high fidelity to nesting rookeries and can use conspecific cues for nest site selection (Reifsneider et al., 2010; experimental manipulation of one rookery indicated that tuatara show no preference for shading, but they do select sites with minimal vegetation and loose soil (Reifsneider et al., 2010).

Using longitudinal behavioral data, we addressed the following questions. (1) Do nesting traits (depth and timing of oviposition) that could be behaviorally modified vary with seasonal (air temperature prior to nesting, air temperature during nesting, oviposition date, or year) or location (rookery) cues? (2) How do variations in the timing of oviposition or nest depth affect outcomes on the incubation environment (nest temperature characteristics, timing, and duration of the thermosensitive period for sex determination, nest success, and sex ratios)? We discuss the ecological consequences of climate warming on a reptile species with TSD when potential behavioral mechanisms affecting the incubation environment and their variability are considered.

2 | METHODS

Nesting activity of tuatara was recorded on Stephens Island (Takapourewa; 40.666°S, 174.001°E, 187 m asl), New Zealand by searching seven known rookeries for nesting attempts during late October to early December, annually from 2002–2005 (described in Reifsneider et al., 2009, 2010). Nesting females were individually marked using passive integrated transponders (PIT tags: Allflex, ISO FDX-B; Reifsneider et al., 2009).
Following completion, each nest was carefully excavated, and oviposition date and a description of each backfilled burrow (i.e., whether it contained soil or vegetation, length of backfill, presence of cavity around eggs) were recorded. A datalogger (Onset Stowaway Tidbit TB132; accuracy to ±0.2°C) was inserted into each nest immediately beside eggs to record hourly temperatures for modeling development rate of embryos, and nests were refilled. Dataloggers were removed by excavating nests approximately 12 months later, and vertical depth from the soil surface to the top egg (or remaining shell), total clutch size and incubation success were recorded. Incubation success was defined as the number of hatched eggs and/or remaining viable eggs, divided by the total number of eggs in the clutch. Nonviable eggs were easily identifiable as hardened, infested by beetles, and/or desiccated. Where available, data from nests investigated in 1998 (Nelson et al., 2004) are also included for comparison.

2.1 Analyses

Using analyses of variance (ANOVA), the following tests were performed to understand influences on nest traits: (1a) the effects of oviposition date and air temperature on oviposition date (maximum, minimum, and mean), were each respectively investigated for influences on nest depth; and (1b) the effect of year, rookery location, air temperature (maximum, minimum, and mean) on the oviposition date, and temperature in the spring leading up to the oviposition date (represented by degree-days), were each respectively investigated for influences on oviposition date. Air temperatures were from the meteorological station on Takapourewa (station network #G04601; agent #4153; obtained from the New Zealand National Climate Database; https://cliffo.niwa.co.nz/). Weather data were not collected in November 2004; therefore, analyses including air temperatures were not conducted for the 2004 nesting season. Degree days, an indication of the relative warmth of the period prior to nesting, were recorded for each year of total temperatures between September 1st and November 1st. Degree days were calculated using daily air temperatures at 9 am; where temperatures were below 10°C, 0°C was added to the total (as embryonic development is estimated to be negligible below 10°C in tuatara; Mitchell et al., 2006), and where temperatures were above 10°C, the cumulative total was increased by the daily temperature minus 10°C (e.g., 11°C resulted in 1°C being added to the cumulative total; Nelson et al., 2004).

Outcomes of the incubation environment were investigated using the following tests: (2a) the effects of oviposition date, nest depth, and year were each respectively investigated for influences on the onset of the thermosensitive period (TSP; the period when sex is determined in TSD reptiles); (2b) nest depth, year, and the timing of the onset of the TSP were investigated for effects on the TSP duration; and (2c) nest depth, year, and rookery location were investigated, respectively, for effects on nest temperature (separate analyses were performed for maximum, minimum, mean, and constant temperature-equivalent (CTE)). For each nest, hourly developmental rates were estimated using a nonlinear development rate function and hourly temperature records from data loggers (Georges, Beggs, Young, & Doody, 2005; Mitchell et al., 2008). Modeled development rates rather than hatching dates were used to calculate incubation duration (Mitchell et al., 2008). Cumulative development was used to calculate the date at which nests reached 30% of development was determined as the onset of the TSP; the number of days nests took to develop from 30% to 35% was recorded as the duration of the TSP (Nelson et al., 2010). The 30 and 35% developmental points were established from incubation treatment switching experiments and their resulting sex ratios (Nelson et al., 2010). The median temperature in nests during the 30–35% developmental window was designated as the CTE (Mitchell et al., 2008; Nelson et al., 2010).

In addition, (2d) incubation success was evaluated with respect to nest depth, year, rookery, and temperature extremes (maximum and minimum for the whole incubation period); and (2e) sex ratios of nests were investigated for effects of nest depth, year, rookery, and nest success. Sex ratios of nests were predicted from CTEs for 2002–2005 (Nelson et al., 2004) using the A-logistic function for determining sex in tuatara (Mitchell et al., 2006), and numbers of each sex were estimated based on the total clutch size (actual numbers of each sex were used for 1998). To account for potential effects of mortality in nests on sex ratios, we also used the number of successful eggs in each nest rather than the total in a second calculation. Mortality may be unrelated to temperature and reflect other random factors (e.g., predation by beetles), and it was therefore worth considering both predictions.

Analyses were conducted using SPSS Statistics 17.0 (SPSS 2008). Individual females were not associated with nests in more than one season due to the small number of nests monitored compared to the large population size, in combination with infrequent nesting by females (every 2 to 4 years), so repeated measures were not required. Data were normally distributed, and summary statistics are presented as mean ± 1 standard error (SE) unless otherwise stated. All data were evaluated for homogeneity and equality of variance.

3 RESULTS

A total of 118 nests were recorded over 5 years in seven rookeries (Table 1; Carter, 2015; Refsnider et al., 2009; Thompson et al., 1996; see Refsnider et al., 2010 for rookery map).

Effects on nest depth (1a): Average nest depth was 111 mm (SE 0.44; range 10–230 mm; n = 118; Figure 1). Nest depth did not vary among years (F4,88 = 0.358; P = 0.838) or rookeries (F6,88 = 0.592; P = 0.736). However, nest depth increased over the nesting season, with deeper nests measured later in the season (F1, 7 = 6.843; P = 0.007; Figure 2A). Air temperature on the day of nesting was a weak predictor of nest depth (F1 = 3.883; P = 0.052; Figure 2B).

Effects on oviposition date (1b): Oviposition dates differed significantly among years (F4,90 = 24.926; P < 0.001; Table 1), but not among rookeries (F4,90 = 1.198; P = 0.315). Females nested earlier, on average, in 1998 and 2005. Oviposition dates were correlated with temperatures preceding nesting (Figure 3; F1 = 6.052; P = 0.091; R2 = 0.66). A warmer spring resulted in earlier nesting. Air temperatures on oviposition dates were significantly warmer during the later parts of each season (F1 = 30.48; P < 0.001; Figure 2B). Only two nests were completed.
### TABLE 1  Summary data for tuatara nests

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<tbody>
<tr>
<td>No. of days 30–35%</td>
<td>9.6 (0.3)</td>
<td>8–14</td>
<td>9.8 (0.3)</td>
<td>7–14</td>
<td>16.2 (0.8)</td>
<td>9–22</td>
<td>10.0 (0.8)</td>
<td>6–20</td>
<td>10.2 (0.4)</td>
<td>8–12</td>
</tr>
<tr>
<td>development</td>
<td>Nest temperature</td>
<td>21.5 (0.2)</td>
<td>2.9–34.4</td>
<td>21.6 (0.2)</td>
<td>16–38.4</td>
<td>18.6 (0.4)</td>
<td>5.4–38</td>
<td>21.1 (0.4)</td>
<td>5.1–38.2</td>
<td>21.3 (0.9)</td>
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<tr>
<td>CTE (during TSP)</td>
<td>22.1 (0.3)</td>
<td>19.6–24.3</td>
<td>22.1 (0.2)</td>
<td>19.1–24.4</td>
<td>19.6 (0.5)</td>
<td>17–25.1</td>
<td>23.7 (0.8)</td>
<td>18–30.2</td>
<td>22.0 (0.5)</td>
<td>20–24.6</td>
</tr>
<tr>
<td>% of males</td>
<td>50 (10)</td>
<td>0–100</td>
<td>66 (7)</td>
<td>0–100</td>
<td>22 (10)</td>
<td>0–100</td>
<td>64 (9)</td>
<td>0–100</td>
<td>42 (15)</td>
<td>0–100</td>
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<tr>
<td>No. of eggs</td>
<td>9.3 (0.6)</td>
<td>3–13</td>
<td>9 (0.6)</td>
<td>1–22</td>
<td>9.2 (0.9)</td>
<td>1–22</td>
<td>9 (0.7)</td>
<td>4–17</td>
<td>64 (0.6)</td>
<td>4–9</td>
</tr>
<tr>
<td>% success</td>
<td>62.2 (7.8)</td>
<td>0–100</td>
<td>58.8 (6.5)</td>
<td>0–100</td>
<td>61.3 (7.3)</td>
<td>0–100</td>
<td>63.9 (8.6)</td>
<td>0–100</td>
<td>61.8 (13.1)</td>
<td>0–100</td>
</tr>
<tr>
<td>Predicted No. of males</td>
<td>6.0 (0.8)</td>
<td>0–16</td>
<td>2.4 (1.0)</td>
<td>0–14</td>
<td>6.2 (1.1)</td>
<td>0–17</td>
<td>2.8 (1.2)</td>
<td>0–9</td>
<td>28 (12)</td>
<td>0–9</td>
</tr>
<tr>
<td>Predicted No. of females</td>
<td>3.0 (0.7)</td>
<td>0–22</td>
<td>6.8 (1.1)</td>
<td>0–22</td>
<td>2.8 (0.9)</td>
<td>0–10.6</td>
<td>3.6 (1.1)</td>
<td>0–9</td>
<td>11.0 (3.4)</td>
<td>0–9</td>
</tr>
<tr>
<td>No. of males (includes hatching success)</td>
<td>4 (0.8)</td>
<td>0–13</td>
<td>3.1 (0.6)</td>
<td>0–13</td>
<td>2.0 (0.9)</td>
<td>0–14</td>
<td>2.9 (0.8)</td>
<td>0–10</td>
<td>11 (0.6)</td>
<td>0–6</td>
</tr>
<tr>
<td>No. of females (includes hatching success)</td>
<td>2 (0.7)</td>
<td>0–10</td>
<td>1.8 (0.6)</td>
<td>0–13</td>
<td>4.1 (1.0)</td>
<td>0–22</td>
<td>2.3 (0.8)</td>
<td>0–9</td>
<td>25 (0.7)</td>
<td>0–6</td>
</tr>
<tr>
<td>Total No. of nests</td>
<td>23</td>
<td>41</td>
<td>23</td>
<td>22</td>
<td>9</td>
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<tr>
<td>Total No. of eggs</td>
<td>214</td>
<td>367</td>
<td>211</td>
<td>198</td>
<td>58</td>
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<td></td>
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<tr>
<td>Total No. of males (includes hatching success)*</td>
<td>92</td>
<td>129</td>
<td>46</td>
<td>65</td>
<td>10</td>
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<tr>
<td>Total No. of females (includes hatching success)*</td>
<td>45</td>
<td>74</td>
<td>95</td>
<td>50</td>
<td>23</td>
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Actual sex ratios were available for 1998; predicted numbers of each sex are presented for other years, incorporating clutch size and hatching success. The thermosensitive period (TSP) occurs between 30 and 35% development. Nest temperatures were measured by data loggers in nests throughout incubation. As nest temperatures vary substantially, we include the controlled temperature equivalent (CTE) for temperatures during the TSP for context relevant to the sex determination period. *In 1998, hatchling sex was verified by laparoscopy, but in other years, numbers of each sex were estimated from modeling outputs using median CTE temperatures throughout the TSP. Sex ratio ranged in every year from 0 to 100% males among nests.
The average depth of tuatara nests (111 ± 0.4 mm) did not vary significantly among years. Numbers in parentheses represent the number of nests, dots are means, and bars indicate the range.

Nest depth increased slightly over the nesting season. Air temperatures fluctuated daily, but were significantly warmer later in the nesting season on oviposition dates (data do not include 2004 nests as air temperatures in November were not available).

Oviposition dates were earlier in years with a warmer spring (measured in degree days above 10°C between September 1st and November 1st for mean oviposition date). Average oviposition dates are presented ± 1SE.

The thermosensitive period (TSP) occurred primarily in late January and February, and over an average 11 days. The TSP was significantly earlier in 1998 and 2005 than in other seasons, and later and longer in 2003. Crosses indicate the beginning of the average TSP for each season (date refers to nesting season) and dashes indicate the end of the TSP, on average for each season.

Maximum temperatures recorded in nests significantly decreased with increasing depth ($F_{1,80} = 14.956, P < 0.001$) but were not affected by year ($F_{4,80} = 1.31, P = 0.273$) or rookery ($F_{6,80} = 2.089, P = 0.064$). Minimum temperatures recorded in nests significantly increased with increasing depth ($F_{1,80} = 37.089, P < 0.001$) and varied with year ($F_{4,80} = 7.756, P < 0.001$), but not rookery ($F_{6,80} = 14.956, P = 0.082$). In 2002, minimum temperatures were significantly lower than in all other years (mean 5.6 ± 0.3°C). Mean nest temperatures and CTEs were significantly affected by depth (mean: $F_{1,80} = 4.364, P = 0.040$; CTE: $F_{1,80} = 7.731, P = 0.007$), year (mean: $F_{4,80} = 14.262, P < 0.001$; CTE: $F_{4,80} = 9.783, P < 0.001$), and rookery (mean: $F_{6,80} = 4.698, P < 0.001$; CTE: $F_{6,80} = 7.283, P < 0.001$). Deeper nests were cooler.
Effects on nest success (2d): Nest success (% hatching) was not affected by nest depth, year, rookery, or minimum or maximum nest temperature. Eggs were exposed to nest temperatures from 1.6–38.5°C.

Effects on sex ratio (2e): Nest depth \( F_{1,104} = 4.34, P = 0.04 \) and year \( F_{4,104} = 4.671, P = 0.002 \) were significant predictors of sex ratio. However, if nest temperature (minimum, maximum, or CTE) was included in models, depth was no longer significant. Predicted sex ratios varied among nests from 0–100% males in all years. Predicted sex ratios including all nests varied among years (Table 1). Sex ratio was significantly influenced by rookery \( F_{4,104} = 2.642, P = 0.02 \). Nest success was not significantly different between predominantly male-producing versus female-producing nests \((64 \pm 3.5\% \); \( F_{1,109} = 0.559, P = 0.456 \)). Therefore, when predicted sexes were multiplied by total clutch size and compared to those including only successful eggs, predicted sex ratios changed only slightly. In particular, nests in 2004 were predicted to become more female-biased, whereas in 2002, there was an increase in the predicted male bias when only numbers of successful eggs were included.

4 | DISCUSSION

Tuatara did not vary their nest depth by rookery location or year. Although slightly deeper nests were observed later in the season, construction may be easier for later-nesting females if they reuse tunnels dug by earlier nesters (Refsnider et al., 2010) or warmer ambient temperatures may allow for increased digging activity, therefore, we cannot confirm this is a behavioral response to thermal cues.

Tuatara constructed nests on rookeries consistent with observations of nesting activity in 1986 (Thompson et al., 1996), and where we know of repeated nesting attempts by individuals, at least in the short term, females returned to sites within 16.5 m of their previous nesting efforts (Refsnider et al., 2010). Even though nest depth did not vary, physical characteristics (such as aspect and soil type; Carter, 2015; Thompson et al., 1996) of rookeries varied, and so eggs laid in different rookeries experienced different thermal environments and produced different predicted sex ratios, with additional year-to-year variation.

Oviposition dates were correlated with austral spring temperatures (September–November), with warmer spring temperatures resulting in earlier nesting. Nesting date is predicted to determine the timing of the thermosensitive period (TSP). Tuatara tended to nest at a time that resulted in the TSP occurring during what is, locally, the warmest period of the year. Average temperature in January and February is 17.2°C, whereas the months of either side are cooler: December temperature average is 15.7°C and March temperature average is 15.6°C (temperatures were annually averaged from 1981 to 2010; https://www.niwa.co.nz/climate/monthly). Temperatures during the TSP are predicted to produce a female bias if the TSP occurs earlier (e.g., 2005) or later (e.g., 2003) than the hottest months [Color figure can be viewed at wileyonlinelibrary.com]

![FIGURE 5 The onset of the TSP currently occurs in the hottest period of the year, in January and February, when average temperatures are 17.2°C (indicated by the dark grey (or red) bar); light grey (or yellow) bar represents cooler months: December average 15.7°C and March average 15.6°C; temperatures are annual averages 1981–2010; https://www.niwa.co.nz/climate/monthly). Temperatures during the TSP are predicted to produce a female bias if the TSP occurs earlier (e.g., 2005) or later (e.g., 2003) than the hottest months [Color figure can be viewed at wileyonlinelibrary.com]](image)

ever, the 4°C or more increase in mean annual air temperatures that is predicted to occur over the next 100 years may override the mediating effect of oviposition date and rookery location on hatchling sex ratios (Carter, 2015).

Successful hatching was not affected by nest depth, year, rookery, or minimum or maximum nest temperatures. Nests reached temperatures well outside the range of those predicted to be successful from artificial incubation regimes (Thompson, 1990). In the short term, tuatara eggs seem robust to variability in nest temperatures from at least 1.6–38.5°C, but the length of time that eggs can survive at the extremes of this range are not known. Extreme climate warming scenarios that increase environmental minimums could result in shorter incubation duration and earlier TSPs, potentially facilitating a female bias in offspring sex ratios. However, increases in maximum temperatures could result in increased mortality of eggs as well as a male-biased sex ratio.

Our research concurs with research on painted turtles, where the timing of nesting was also correlated with winter temperatures (Schwanz & Janzen, 2008). However, modeling of advancing nesting dates in painted turtles, which have a sex determination pattern in which females are produced from warmer temperatures, predicted production of 100% female offspring and increased mortality (Telemeco et al., 2013).

For egg-laying reptiles, nest-site choice is hypothesized to be driven primarily by the need to maximize embryonic survival and, secondarily, to optimize offspring phenotype for the environment, thereby increasing fitness of mothers and their offspring. Natal philopatry may be important as long as nesting locations remain suitable, and imperfect natal homing might better represent a successful long term system (Refsnider & Janzen, 2010). Our results provide empirical evidence consistent with this hypothesis, in that the consistency in nest depth across rookeries and years, and the natal philopatry observed in the short term in tuatara, are adaptations for maximizing survival of eggs.
However, understanding what drives any variation in nest depth and the timing of oviposition, in conjunction with site-specific thermal information, will inform not only predictions of nesting success but also important phenotypic consequences for surviving hatchlings, such as sex ratios and performance characteristics, in addition to effects of climate warming and likely success of management actions (e.g., translocations; Besson, Nelson, Nottingham, & Cree, 2012).

Phenotypic consequences are particularly pertinent for reptiles with TSD, as populations with successful production of only one sex are effectively functionally extinct. Potential drivers of nest depth and timing of oviposition include a deliberate response (i.e., choice) to thermal cues, a physiological response driven by increased activity, a function of female size (for nest depth, specifically, with potential to change as females increase in size and age), and/or an independent female characteristic (potentially repeatable through years and sites). Tuatara activity is strongly associated with temperature (e.g., Vermunt, Hare, & Besson, 2014), so readiness to lay and nesting activity on rookeries is likely to be physiologically correlated with temperature. Deliberate behavioral responses to thermal cues by tuatara seem unlikely based on a small shading experiment (Refsnider et al., 2010), but this contrasts with the well-studied painted turtle, where choice of shading at nest sites was responsive to cues at nesting (Refsnider & Janzen, 2012), so further research is needed. Nest depths in painted turtles also vary among populations throughout their range (Refsnider & Janzen, 2012), whereas our research was conducted on the only island where we could have obtained sufficient data for comparison of nesting across years due to the large population size. In a smaller population of tuatara on nearby North Brother Island, limitations on nesting habitat is likely contributing to the extreme male bias in that population, threatening its population viability. Effectively, tuatara only have one available nesting environment on North Brother Island, which is currently male-producing (Grayson et al., 2014; Mitchell et al., 2008).

We know little about nesting in other tuatara populations, particularly those in different ecological regions, e.g., whether thermal environments in those locations could drive different nesting behavior in tuatara. Northern populations of tuatara may experience mean annual air temperatures that are 4°C warmer, on average, than those on Takapourewa (https://cliflo.niwa.co.nz/). Females in some populations reach larger sizes (Cree, 2014), and nesting dates known only from captivity indicate a less specific nesting season (Nelson unpublished data), leaving enormous breadth for future research (on albeit smaller datasets) to understand the potential for individual tuatara to behaviorally offset increasing temperatures when nesting in the future, the role of habitat variability in maintaining production of both sexes, and the variation among populations in nesting behavior, particularly with respect to the effects on oviposition date and therefore the timing of the thermosensitive period. We have some confidence that tuatara can respond over time to changing nesting opportunities, since current nesting locations on Takapourewa would have been unsuitably cool for successful incubation when the island was predominantly forested ~120 years ago (Cree et al., 1989; Dendy, 1899; Dieffenbach, 1843).

Nest depth in reptiles is often associated with female size (e.g., painted turtles; Refsnider & Janzen, 2012). However, female size (and by inference, age) of tuatara is unlikely to determine nest depth, because nests are constructed as burrows into slopes; thus, the length of females should have little bearing on the depth of undisturbed soil above eggs at the end of very long burrows (e.g., 1 m length burrows may be constructed by females but nest depth may be still only 110 mm; Nelson unpublished data). However, further research is required to investigate whether individual females nest consistently. For example, understanding whether individual females nest more deeply or habitually earlier would provide evidence for the potential for heritability of nesting characteristics and contribute to the understanding of the development, maintenance, and/or switching of sex-determining systems through evolutionary time and the survival of this lineage through previous climate change episodes.

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ORCID

Nicola J. Nelson https://orcid.org/0000-0002-1641-6030
Jeannine M. Refsnider https://orcid.org/0000-0001-5154-4356
Anna L. Carter https://orcid.org/0000-0003-4392-277X

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