Is cool egg incubation temperature a limiting factor for the translocation of tuatara to southern New Zealand?

Anne A. Besson[1, *], Nicola J. Nelson[2], Cathy M. Nottingham[1] and Alison Cree[1]

[1]Department of Zoology, University of Otago, PO Box 56, Dunedin 9054, New Zealand
[2]Allan Wilson Centre for Molecular Ecology and Evolution, School of Biological Sciences, Victoria University of Wellington, New Zealand
[*]Corresponding author. Email: anne.besson@gmail.com

Published on-line: 8 November 2011

Abstract: Conservation strategy for maintaining and protecting tuatara (*Sphenodon punctatus*), a rare endemic reptile from New Zealand, includes the reinstatement of populations through the past historical range. A proposal exists to translocate tuatara from Stephens Island in Cook Strait to the Orokonui Ecosanctuary (Te Korowai o Mihiwaka), a coastal site in southern New Zealand. The proposed site is within the former latitudinal range of the genus, but lies outside the current distribution of tuatara, where the climate is warmer. In this study, we examined whether cool incubation temperature is a limiting factor for the proposed reintroduction of tuatara to Orokonui. The tuatara is a species with temperature-dependent sex determination, with only females being produced at low incubation temperatures. Thus, cool southern temperatures may produce only females, even if incubation temperatures are high enough to support successful development. We experimentally translocated tuatara eggs to the ecosanctuary and found that nest temperatures were consistently below those of nests in their current natural distribution and would produce only female hatchlings if successful incubation occurred. An addition of sand to soil did not raise temperatures sufficiently to produce both sexes. However, additional assessments of soil temperatures in a third year indicated that some new sites were warm enough for males to be produced. Given that other aspects of site suitability appear favourable, and that global temperatures are predicted to rise in the near future, which should produce a more viable incubation environment, translocation of this long-lived reptile to the southern ecosanctuary is worth further exploration. However, monitoring of female nesting behaviour, including nest locations, depths and resulting temperatures, will be essential. Our study demonstrates an experimental approach for assessing site suitability for translocation that may be relevant to other egg-laying reptiles.

Keywords: conservation; egg relocation; temperature-dependent sex determination; *Sphenodon*

Introduction

Translocation of animals is a common practice in conservation programmes, for example to reintroduce species that have become either locally or globally extinct in the wild (Armstrong & Seddon 2008). In New Zealand, the main threat to native biodiversity is introduced mammals, but the successful removal of mammals from islands around New Zealand has enabled translocation of reptiles to areas that were part of their historical range (Towns & Ferreira 2001). About 80 translocations of reptiles have occurred in New Zealand since 1985 (Sherley et al. 2010). Although most species are long-lived and further post-release monitoring will be required to confirm self-sustaining populations, the majority of translocations of reptiles to rodent-free sites have shown provisional indicators of success (Nelson et al. 2002; Towns et al. 2007).

Tuatara (*Sphenodon punctatus*) are endemic to New Zealand, and of high conservation significance and international interest (e.g. Lowe et al. 2010) as the sole living representative of Order Rhynchocephalia. Tuatara were once widespread over both the North and South Island approximately 1000 years ago (36–46° S; Cree & Butler 1993; Towns & Daugherty 1994) but are now naturally restricted to about 32 offshore islands in the Cook Strait region and northern New Zealand (36–41° S) due to habitat destruction and introduced predators (Fig. 1a). So far, tuatara have only been reintroduced to sites essentially within the latitudinal range of current populations (36–41° S, Fig. 1a).

A proposal exists to translocate tuatara from Stephens Island in Cook Strait to the Orokonui Ecosanctuary (Te Korowai o Mihiwaka), a coastal site in southern New Zealand, near the city of Dunedin (45° S; Fig. 1a). The proposed site is within the former latitudinal range of the genus, and about 7 km from a midden deposit containing tuatara bones (Hamel 2001), suggesting that until a few hundred years ago tuatara occurred in the general vicinity. The translocation of tuatara to Orokonui would fit the long-term goal of the Tuatara Recovery Plan, which is to reinstate 'wild populations of tuatara throughout their pre-human range as components of healthy ecosystems' (Gaze 2001). However, Orokonui lies outside the current distribution of *Sphenodon punctatus*, and its climate is on average c. 6°C cooler (NIWA National Climate Database, http://www.niwa.cri.nz.ncc). Tuatara appear to have an uncommon form of temperature-dependent sex determination (TSD), FM or type IB, in which females result from cool egg incubation temperatures and males from warm ones (Mitchell et al. 2006). Thus, cool southern temperatures could potentially limit the production of males. Furthermore, whether extinct mainland populations were genetically identical with current populations is unknown. It is possible that tuatara that lived in southern New Zealand had special adaptations to cool temperatures. For example, the pivotal temperature (temperature at which

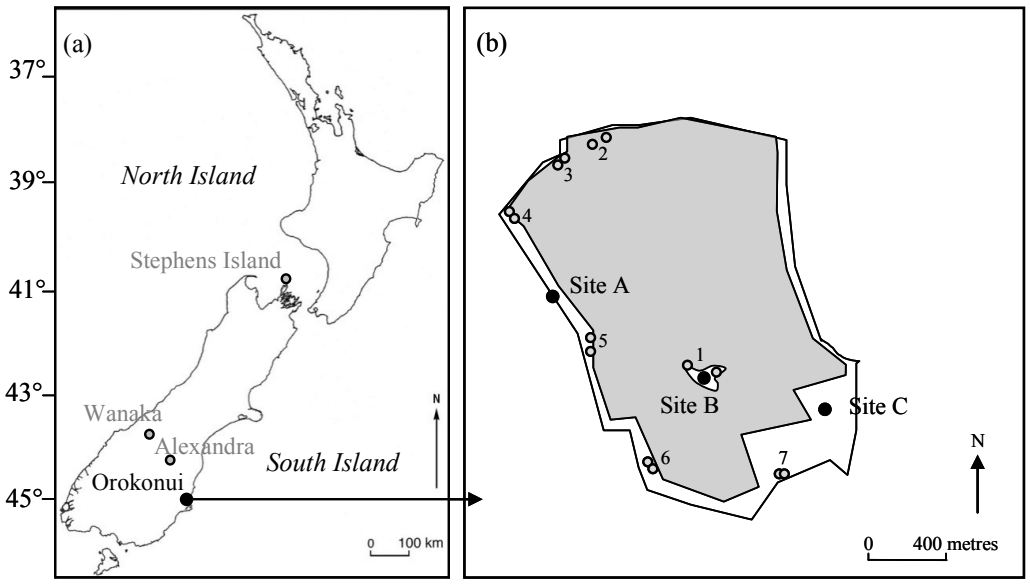

Figure 1. (a) Map of New Zealand showing the main locations and latitudes cited in the text. (b) Sites at Orokonui Ecosanctuary, South Island, where soil temperature was monitored during the first year (2006, sites A, B and C), where eggs were incubated in the second year (2007, site A), and where soil temperatures were monitored in the third year (sites 1–7, grey dots). Forested areas are grey on the map and open areas are white.

hatchling sex ratio is 1:1) may have been lower for extinct southern populations compared with existing northern ones, or the thermosensitive period (TSP: period during embryonic development when sex is determined) may have been earlier or later during the year. However, most studies have failed to show variations in pivotal temperature or other reproductive variables between turtle populations despite differences in the thermal environment (Bull et al. 1982; Mrosovsky 1988; Ewert et al. 2005). Pivotal temperature appears not to vary by more than a degree among three populations of tuatara studied (Mitchell et al. 2006).

The objectives of the present study were to determine whether incubation of tuatara eggs in southern New Zealand would be successful and if both sexes could be produced. To test this, we performed an experimental translocation of tuatara eggs to Orokonui and estimated hatching success and sex ratio. The following season, we monitored soil temperatures of further potential nesting sites in unmanipulated soil to estimate the sex ratios that would be produced. We use these results to assess the likelihood that tuatara translocated to southern New Zealand would be able to nest successfully and produce hatchlings of both sexes.

Methods

Nest characteristics of tuatara

The nesting season on Stephens Island (Takapourewa), Cook Strait, occurs from late October until early December (Thompson et al. 1996). Tuatara nest in rookeries in open areas, as temperature under full forest cover is too low for embryonic development (Thompson et al. 1996). Nests consist of a tunnel sloping downward from the entrance, and mean depth from the top egg to the soil surface is 107 mm (range 10–230 mm; Nelson et al. 2004a). In some nests, eggs are contained in an air-filled chamber, but in others, eggs are lodged against the ceiling (Thompson et al. 1996; NJN, pers. obs.). Mean clutch size is 9.4 eggs (range 1–18 eggs) and eggs are deposited in 1–3 layers (Newman et al. 1994). After egg-laying, the nest is backfilled with soil and sometimes filled by the female with grass and other material found near the nest entrance. On Stephens Island, female tuatara nest once every 2–5 years (Cree et al. 1992) and show high fidelity to nesting rookeries (Refsnider et al. 2010). Incubation takes 11–16 months to complete (Thompson & Daugherty 1992), exposing eggs to both seasonal and annual fluctuation in temperatures. Incubation temperature in successful nests throughout the year ranges from 1.6° to 38.4°C and nest temperatures fluctuate daily by 0.5–15° C (Nelson et al. 2004a). Hatching success from natural nests on Stephens Island was 48% in one year (Thompson et al. 1996); causes of incubation failure include desiccation, and nest destruction by other females (Refsnider et al. 2009).

Egg incubation, hatching success and sex ratio

To select a suitable site and depth for egg incubation, we first monitored soil temperatures across 10 months (January–October 2006) at three sites (Site A140 m a.s.l.; site B 220 m a.s.l.; site C 320 m a.s.l.) and two depths (100 mm and 200 mm) at Orokonui Ecosanctuary (Fig. 1b). Overall results showed that sites A, B and C were cooler than natural nests on Stephens Island (data from Nelson et al. 2004a; Table 1), but remained within a temperature range that could be conducive to embryonic development. Even at the warmest site and depth (site A, 100 mm), Orokonui temperature profiles from 2006 were cooler than natural cool nests on Stephens Island that produced female tuatara. Thus, all potential nest sites monitored at Orokonui in 2006 would be expected to produce females. Additional monitoring at a shallower depth (50 mm) at site A from June to October 2006 revealed soil temperatures up to 3°C warmer than at 100 mm depth. Based on these results, we chose to incubate translocated eggs at site A. Eggs were incubated at 50 mm depth (with addition of sand; see below) for the warm treatment and at 100 mm depth for the cool treatment, thus maximising the chance of producing tuatara of both sexes.

To test the predicted sex ratio, and determine if incubation would be successful, we translocated viable tuatara eggs in the second season to artificial nests (hereafter nests) at Orokonui. Eggs were collected from Stephens Island, the preferred source

Table 1. Range of temperatures, constant temperature equivalents (CTE) and time spent above 11.1°C and 22°C for natural successful nests of tuatara (*Sphenodon punctatus*) on Stephens Island, and for artificial nests at Orokonui Ecosanctuary, New Zealand.

		Temperature (°C)			No. hours	No. hours	CTE values °C (% of males)			
Sites	*n*	Mean	Min.	Max.	>11.1°C	>22.0°C	0.15	0.20	0.25	0.30
Natural nests[1]										
Warm nests	26	15.0–17.0	1.6–8.2	25.2–38.4	7216 ± 103	1035 ± 48				
Cool nests	14	14.0–16.2	3.8–8.0	20.0–38.2	7218 ± 173	467 ± 89				
Artificial nests[2]										
Warm 'nests'	4	11.1–11.3	1.8–2.3	25.8–27.2	4208 ± 31	99 ± 12	17.6 (0)	16.0 (0)	11.3 (0)	16.8
Cool 'nests'	4	9.5–9.8	2.2–2.6	20.1–24.5	3317 ± 51	0.8–0.5	13.9 (0)	[3]	[3]	[3]

[1]Data from Nelson et al. (2004a). Temperature recorded from December 2002 to November 2003 on Stephens Island.
[2]Temperatures recorded from December 2006 to December 2007 at Orokonui for nests with translocated eggs.
[3]CTEs could not be calculated as all the eggs from the cold nests failed following a heavy flood.

population for translocations because of high abundance and genetic diversity (Miller et al. 2007). Newly laid eggs were collected from or near natural nests in November 2006 (n = 110, including six from disturbed nests as per our permit conditions). We estimate that the collection had little impact on the resident population of tuatara on Stephens Island. Sixty juveniles (the predicted number that might have hatched from the eggs if incubated in nests on Stephens Island) represent only about 0.2% of the population of tuatara there, which is estimated at 30 000 – 50 000 individuals (Newman 1982) and probably only a small proportion of these would have contributed to the breeding population.

Eggs were transported in damp vermiculite by air to Dunedin and then by car to the University of Otago, where they were placed in an incubator at 18°C while more eggs were collected. Fourteen eggs failed within 2 weeks of collection. Of the remaining 96 eggs, 88 were incubated at Orokonui and eight were incubated in controlled-temperature incubators at 18°C (female-producing temperature, n = 4) and 24°C (male-producing temperature, n = 4). Laboratory-incubated eggs provided a control for the effects of early transportation on egg incubation success, and were incubated following the procedure of Nelson et al. (2004c).

On 11 December 2006, 3–4 weeks after egg collection (which corresponds to less than 6% of embryonic development, and precedes the period when sex is determined; Nelson et al. 2010), 44 eggs were buried at Orokonui in four 'cool nests' at 100 mm depth in natural soil; the remaining 44 eggs were buried in four 'warm nests' at 50 mm depth in an equal mix of horticultural sand and natural soil (n = 11 per clutch; see below for rationale and significance of adding sand to soil). We mixed the clutches to avoid any parental effect on hatching success of nests, and verified that clutches in all nests had similar mass ($F_{8,87} = 0.52$, $P = 0.83$). During incubation, eggs were protected by 30 × 30 × 30 cm cages (made of stainless steel, mesh size of 6 × 25 mm, http://www.xcluder.co.nz) to minimise the risk of predation or damage, since introduced mammals (such as pigs and rodents, which are potential nest predators) were not yet exterminated from the sanctuary. Inside each nest, the eggs were positioned in a single layer, without contact with each other, in a radial arrangement. Temperature inside each nest was recorded every hour by a data logger (HOBO pendant©) placed 10 mm in front of the eggs. One nest from each treatment (cool and warm sites) was partially excavated in March 2007 to determine whether any eggs had survived the first summer. To minimise disturbance, we carefully opened the nests from the side to observe at least five eggs out of the 11 present in each nest. In the warm nest, five eggs out of five appeared turgid and in good condition. In the cool nest, three eggs out of five appeared in good condition, but two others had collapsed and were removed from the nest. The cool site became waterlogged in July 2007 after heavy rain (see Results) and all the eggs at this site failed. One egg from the warm site was retrieved in September 2007, and the removed embryo was euthanized by halothane vapour and dissected to determine whether embryonic development was sufficiently advanced to be past the sex determination period. The egg had a well-differentiated embryo (early stage R on the scale of Dendy (1899), equivalent to stage 38 on the Dufaure and Hubert scheme (1961) for *Lacerta vivipara*; Moffat 1985). Histology revealed that the gonads were just starting to differentiate (probable ovaries). At the end of November 2007, another embryo from the warm site was dissected (middle stage R-stage 39) and ovaries were clearly differentiated. The eggs from the warm site were therefore assumed to be past the sex-determination period.

Eggs were retrieved 2 weeks later, after the expected time of sex determination and before the expected time of hatching. Eggs were taken to the University of Otago to complete incubation at 22°C or to be dissected if they had failed. Data loggers were left with the eggs, during transit and in the incubator, to provide a complete set of incubation temperatures. Hatchling mass, snout–vent length (SVL), head width, and tail length (TL) were recorded when the allantoic sac fell off, c. 2–3 days after hatching. Each hatchling was given a unique toe-clip for identification. Hatchlings were reared in individual plastic containers (17 × 28 × 43 cm) containing a mix of horticultural sand, bark mulch and topsoil, a water dish, and basking and retreat sites. Hatchlings were fed twice a week with crickets or locusts dusted with vitamin–mineral supplement; water was freely available. Juvenile tuatara cannot be sexed externally with confidence until they attain c. 130–160 mm SVL. Laparoscopy of internal reproductive organs was conducted in December 2008 at the University of Otago (see Nelson et al. 2004b for methods) on the four largest hatchlings (controls from lab incubations); the sex predicted by the constant incubation temperature (18°C and 24°C) was confirmed in each case.

For the field incubations, sand was added to the soil in the warm nests in the hope of increasing soil temperature sufficiently to produce male tuatara. A retrospective test was conducted from January to March 2008 to determine whether addition of sand increased soil temperatures as anticipated and also to verify whether cages had any effect on soil temperatures. We measured soil temperatures in the five empty nests from the warm site (containing soil and sand and surrounded by a cage), as well as in five plots of the same size containing only soil (no cage), and in five plots containing soil and sand (no cage). These north-facing nests were all within an area 2.0 × 3.5 m. Temperature inside each nest was recorded every hour by a data logger (HOBO pendant©).

New potential nesting sites

New potential nesting sites (open, sunny and north-facing, but close to forest) were created prior to the third season by the sanctuary managers as part of habitat restoration programmes. To determine if these new sites would be warm enough to produce male tuatara, we monitored soil temperatures from November 2008 to March 2009 (late austral spring to end of the austral summer, the period when sex is likely to be determined). We measured soil temperatures by placing data loggers (iButton and HOBO pendants©) at 50 mm depth in the ground at seven sites ranging from 31 to 314 m a.s.l. (Fig. 1b). Two loggers placed within a few metres of each other at each site recorded soil temperature every hour.

Estimation of sex ratio

To estimate the sex ratio produced by a given soil temperature profile, we converted soil or nest temperatures into a constant temperature equivalent (CTE; Georges 1989). In calculating CTE, the model takes account of the variance in temperatures, providing a more meaningful representation of the nest environment than the mean temperature (Nelson et al. 2004a). The CTE for each nest or potential nest was converted into a sex ratio according to the equation from Mitchell et al. (2006):

$$\text{Sex ratio} = (1 + (2^{e3.6} - 1)\exp(1/0.0096 \times (22 - \text{CTE})))^{-1/e3.6}.$$

This sex ratio function is the best fit to the sex ratio data produced from constant incubation experiments on *Sphenodon punctatus*, and has a pivotal temperature (sex ratio 1:1) of 22°C and a transitional range of temperatures ($TRT_{0.05}$) where both sexes are produced, of 1.1°C (Mitchell et al. 2006). We therefore assumed that mixed-sex nests would result for sites with CTE values within the boundaries of $TRT_{0.05}$ (21.2–22.25°C), and that only females would be produced below 21.2°C and only males above 22.25°C (Mitchell et al. 2008). Although the exact timing is unknown in tuatara, the latest information indicates that the thermosensitive period has ended within the first 28–39% of the full incubation period (Nelson et al. 2010). We therefore calculated CTE at 15, 20, 25 and 30% of development. The relationship between temperature and development rate is approximately linear between 15°C and 25°C (Mitchell et al. 2006), but because tuatara embryos can survive brief temperature exposure in natural nests between 1°C and 38°C, a non-linear function was used to estimate the developmental rate of tuatara (Mitchell et al. 2006). Briefly, the inputs used for the Dallwitz Higgins model (Georges et al. 2005) were: maximum developmental rate: $b_1 = 0.98545$; temperature at which developmental rate falls to $b_1/10$: $b_2 = 13.7864$; the temperature at which developmental rate is maximum: $b_3 = 30$; the developmental rate approaching zero at high temperatures is controlled by $b_4 = 6$, and finally, the asymmetry of the relationships is controlled by $b_5 = 0.4$ (refer to Mitchell et al. (2008) for more details on the development rate function).

Statistical analysis

Soil and nest temperatures were analysed using factorial ANOVA, with soil temperature including mean, minimum and maximum as dependent variables and depth and location or treatment as predictors. To compare morphology of hatchlings from natural and artificial incubation, we used ANOVA to compare SVL, and then ANCOVA with body mass, total length and head width as dependent variables and SVL as a covariate. The assumption of normality was verified with the Kolmogorov–Smirnov test and the sphericity assumption with the Mauchley test. Statistical analyses were performed using the software Statistica 6.0 (©StatSoft Inc.). Significance was accepted at $P < 0.05$.

Results

Egg incubation, hatching success and sex ratio

Nest temperatures in the warm and cool sites were monitored at Orokonui from December 2006 to December 2007, a full year of incubation. Temperatures stayed within the range recorded for successful nests on Stephens Island (1.6–38.4°C; Table 1). Nest temperatures at Orokonui were, on an hourly basis, 0° to 6°C lower than mean nest temperatures recorded for natural nests on Stephens Island, regardless of season (Fig. 2). During the night, the difference was sometimes as large as 12°C. Warm nests spent significantly more time above 11.1°C (temperature above which embryonic development begins) during the entire incubation period than the cool nests ($Z = -2.3$, $P = 0.02$, Table 1). Cool nest temperatures at Orokonui remained below 11.1°C for 5 months out of 12, whereas the warm nests remained below 11.1°C for only 3 months.

Annual air temperatures at Orokonui during the year of egg incubation were near the long-term average for the region (0% departure from normal year), whereas annual rainfall (599 mm) was 75% of normal year. During heavy flooding on 30 July 2007, the Orokonui region received a typical month's rainfall (122 mm) within 24 h (Fig. 3; NIWA, National Climate Data Base, http://www.niwa.cri.nz/ncc). Nests at the cool site became waterlogged, and when excavated in December 2007, all had failed. At the warm site, 8 out of 44 eggs, all from the same nest, had survived. Temperature profiles at this nest appeared no different from the three other warm nests that failed. Eggs that failed to hatch were opened and examined for presence of blood vessels and/or traces of embryo. One-third (33%) of the eggs incubated in the warm nests showed signs of embryonic development (pigmentation or eye visible, but no yolk residue), suggesting recent failure, whereas only 2.5% of the eggs coming from the cool nests had traces of embryos.

After excavation in December 2007, the eight viable eggs from warm nests were transferred to an incubator at 22°C (Fig. 3). Seven of these eggs (18% of those set in warm nests) hatched 3 months later, and five hatchlings were viable. Overall (field + lab), incubation took 487 days ± 3 ($n = 7$). One embryo that failed to hatch was at the final stage of embryonic development (stage S; Dendy 1899) when the egg was opened. Another

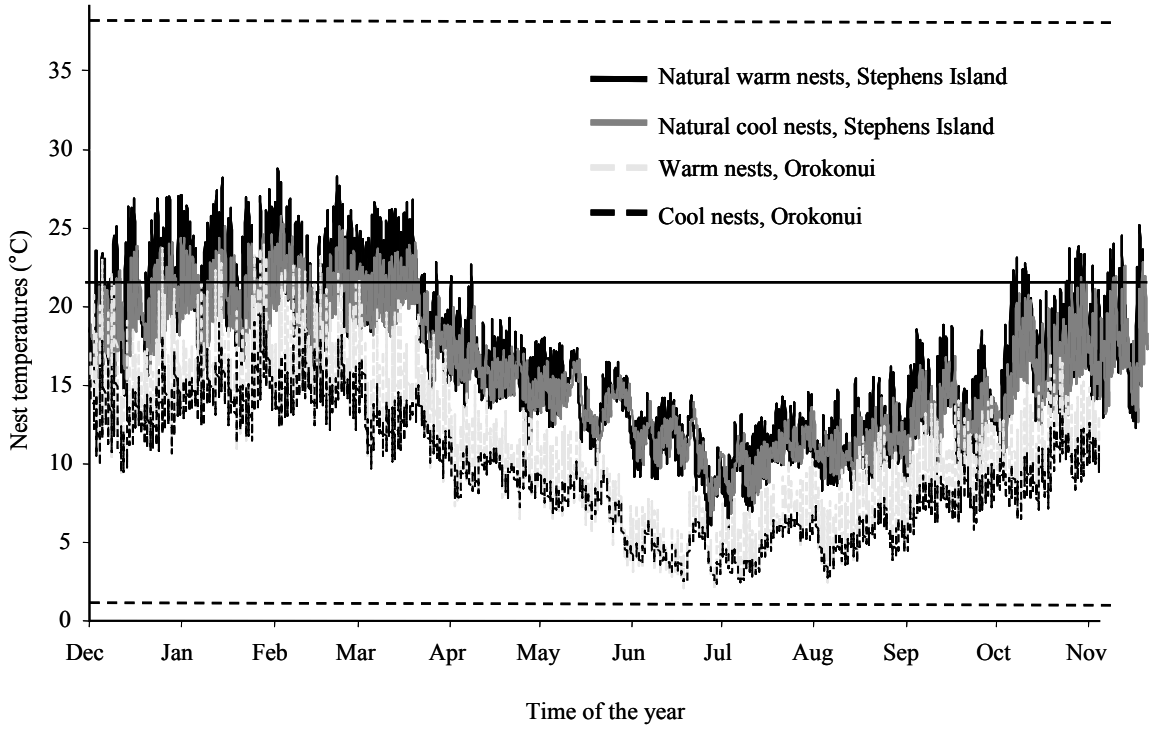

Figure 2. Daily average temperatures for warm and cool artificial nests at Orokonui ($n = 4$ nests with translocated eggs for each subsite, from December 2006) compared with natural nests on Stephens Island ($n = 24$ warm and $n = 16$ cool, in 2002–2003). The dashed horizontal lines represent the range of temperatures within which successful nests remained on Stephens Island (1.6–38.4°C). The black horizontal line shows the pivotal temperature (22°C).

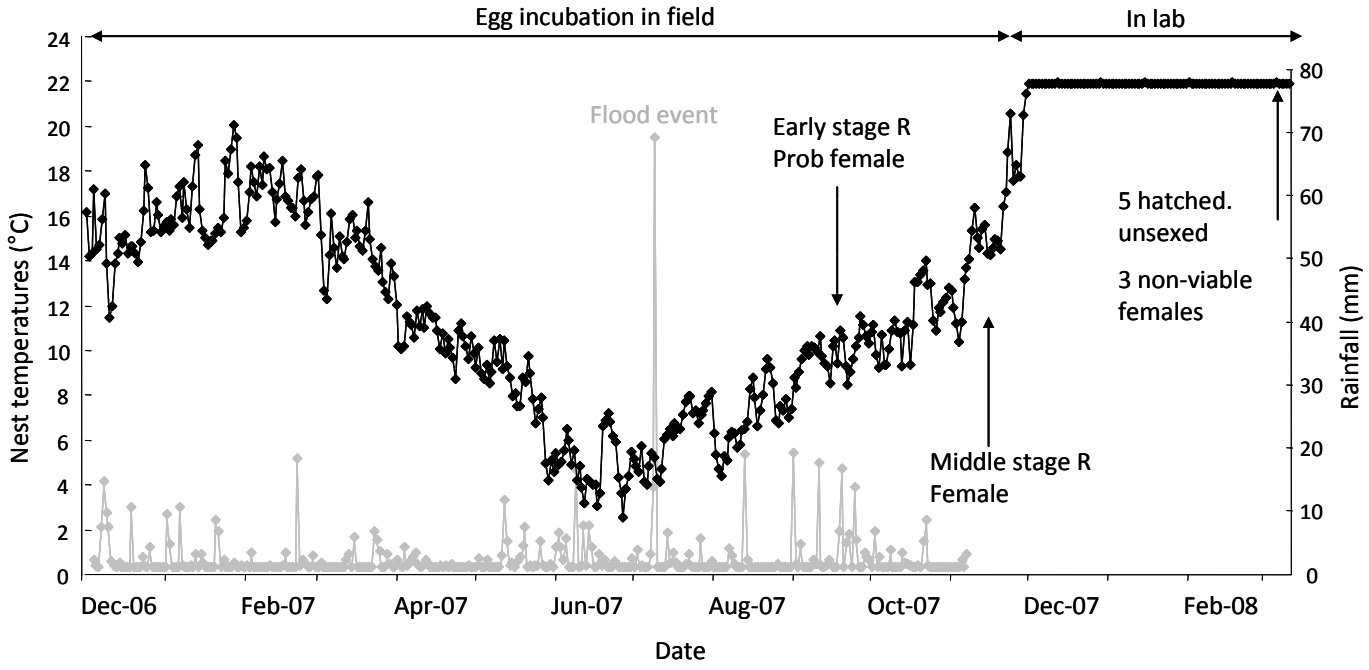

Figure 3. Temperature (daily averages, dark line) of the successful artificial nest with translocated eggs at Orokonui and rainfall data (grey line) for the same year showing the flood event in July. All dissected embryos and non-viable hatchlings were female. The five unsexed hatchlings were too small to be sexed by laparoscopy but the predicted sex (based on soil incubation temperature) was female.

hatchling had uncoordinated and spasmodic movement that impaired its mobility (it was also euthanized). A third that was unable to feed by itself was euthanized after it had lost 20% of its body mass. These three non-viable hatchlings were all females (ovaries and mullerian ducts well developed). The control eggs that were incubated at constant temperature took on average 132.3 ± 3.3 days to hatch at 24°C and 253.3 ± 1.3 days at 18°C. Hatching success was high for these artificially incubated eggs (87.5%). Hatchlings from natural and artificial incubation had similar SVL ($T = 0.05$, $P = 0.96$), and also did not differ in total length ($F_{1,11} = 0.006$, $P = 0.98$), mass ($F_{1,11} = 0.98$, $P = 0.35$) or head width ($F_{1,11} = 3.22$, $P = 0.09$) when adjusted for variation in SVL (Table 2).

As CTE values for Orokonui warm nests during the first third of development (11.3 – 17.6°C; Table 1) were below the threshold for production of males (21.2°C), only females would be expected. Furthermore, the warm nest temperatures remained above 22°C for only 98.7 ± 12.1 h during the entire incubation period and the cold nests spent virtually no time above that value (0.8 ± 0.5 h). Natural nests on Stephens Island that produced males spent on average 1000 h during incubation above 22°C (Table 1).

The sand (added to the warm nests prior to incubation in the hope of increasing soil temperatures) and cages had at most only small effects on soil temperatures. The effect of time of day on mean daytime temperatures varied with treatment (soil alone vs soil+sand vs soil+sand+cage) in January (time of day × treatment, $F_{30,180} = 3.7$, $P < 0.001$), February ($F_{28,168} = 3.4$, $P < 0.001$) and March ($F_{26,156} = 4.3$, $P < 0.001$). The interaction between time and treatment was also significant at night ($3.18 < F < 3.5$, $P < 0.001$). The main effect was that the soil+sand+cage treament (and to a lesser extent the soil+sand treatment) had slightly more rapid heating and cooling than soil alone (i.e. soil alone showed more of a 'lag'). However, the magnitude of the differences in range was small. For example, daily maximum temperatures did not differ among the three treatments in January ($F_{50, 300} = 0.90$, $P = 0.66$) and February ($F_{56, 336} = 0.64$, $P = 0.97$), and the small difference observed in March ($F_{60, 360} = 2.47$, $P < 0.001$) amounted to soil alone being no more than 0.6 ± 0.1°C warmer than the other two treatments.

New suitable incubation sites

During summer of the final year (November 2008 to March 2009, i.e. spanning the period when sex is likely to be determined), we calculated predicted sex ratios from soil temperatures at 50 mm depth from loggers at seven new open sites within the sanctuary (Fig. 1b). Records were complete for 10 loggers, partial for three loggers and unavailable for one logger. For clarity, CTEs are shown for only the three warmest and three coolest loggers with complete traces (Fig. 4). During the warmest period between late December and early February, eight or more loggers (at least 67%) had CTEs within or above the range that would produce at least some males if the TSP occurred during this period. Only two loggers at one site had CTEs below the transitional range throughout the summer (i.e. only females would be expected). There was no significant relationship between CTE and altitude during the warmest period ($P = 0.11$, $N = 13$) nor between mean CTE (calculated for the whole summer) and altitude ($P = 0.12$, $N = 10$). In general, the warmest loggers were at low-altitude sites 3 and 4 (Fig. 1b), which remained free of ground vegetation throughout the summer, and the coldest were at site 1, a higher-altitude site that became substantially shaded by ground cover during the summer.

During the period of observations, summer air temperatures in the Otago region were above average in January (+1.4°C departure from normal year), below average in February (−2.0°C) and near average in March (−0.4°C; NIWA, National Climate Database, http://www.niwa.cri.nz.ncc).

Discussion

Incubation of tuatara eggs at Orokonui led to partial success. One nest out of four at the warm site produced viable hatchlings following transfer of eggs to the laboratory for the final 3 months of incubation. The cages protected the eggs effectively as no evidence of predation was detected and all eggs were retrieved. The cages and the sand did not seem to have a detrimental effect on the eggs and did not increase the soil temperatures enough to produce male offspring. On

Table 2. Incubation time, morphometrics and predicted sex of hatchling tuatara (*Sphenodon punctatus*) from warm artificial nests at Orokonui Ecosanctuary, and from constant incubation in the lab at 18°C and 24°C. Mean ± SE are shown.

Incubation regime	No. incubated	No. hatched	Incubation time (days)	Morphology of viable hatchlings (mm)				Predicted sex
				Snout–vent length	Tail length	Mass	Head width	
Warm nests at Orokonui	44	7[1]	487 ± 3[2]	50.71 ± 1.68	53.07 ± 1.40	4.33 ± 0.20	11.73 ± 0.18	Female
Lab, 24°C constant	4	3	132.3 ± 3.3	51.35 ± 1.32	54.96 ± 1.56	4.68 ± 0.36	11.40 ± 0.19	Male[3]
Lab, 18°C constant	4	4	253.3 ± 1.3	50.36 ± 1.24	51.83 ± 1.25	4.70 ± 0.33	11.63 ± 0.22	Female[4]

[1]Seven out of eight eggs hatched but only five survived.
[2]Incubation time includes 99 ± 1.63 days at 22°C in the lab.
[3]Sex of the three hatchlings was confirmed as male by laparoscopy.
[4]Sex of the largest hatchling was confirmed as female by laparoscopy.

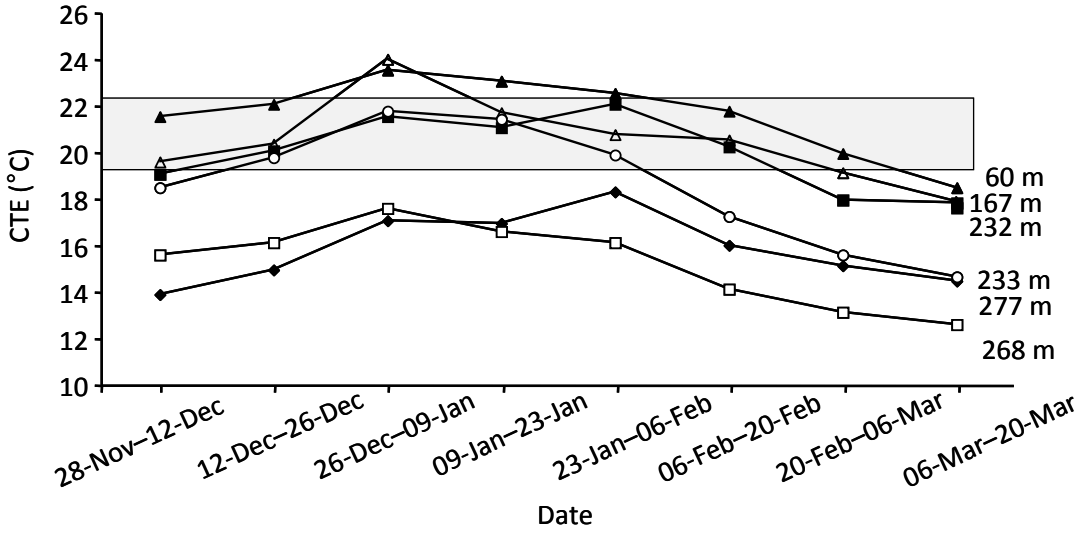

Figure 4. Constant temperature equivalents (CTEs) estimated over 2-week periods during the austral summer (December 2008 – March 2009) for soil at the three warmest and three coldest of 10 loggers with continuous traces at the Orokonui Ecosanctuary (altitudes are given in metres above sea level). The shaded bar shows temperatures predicted to produce mixed-sex clutches. Temperatures above the bar would be male-producing and temperatures below the bar would be female-producing, assuming that the thermosensitive period occurs during this time.

Stephens Island, desiccation is thought to be the principal cause of embryonic mortality (Thompson et al. 1996). Low rainfall at Orokonui during this experiment (75% of normal year) may have contributed to the poor hatching success observed in the warm site (18%). In a previous study on egg incubation in tuatara using 'human-dug nests' on Stephens Island, hatching success was also low (16.4%) and attributed to desiccation (Cree et al. 1989). In our study, the null hatching success at the cold site could have resulted partly from low temperatures, but would almost certainly have eventuated in any case from flooding that occurred in July 2007, when nests became waterlogged.

Along with the possibility of low hatching success and a biased sex ratio, incubation of eggs at Orokonui will take longer (at least two summers) than on Stephens Island as temperatures over a year are cooler than in current natural habitat. Note that the naturally incubated eggs finished incubation under constant 22°C, which would have resulted in earlier hatching than if eggs had been left in nests at Orokonui. Previous studies have shown that for lizard species, the disadvantage of late hatching is that hatchlings experience less favourable environmental conditions and thus are unable to grow as quickly and store as much fat as hatchlings that emerge early in the season (Olsson & Shine 1997). In addition, hatchlings from cool incubation are usually larger due to more yolk material converted into tissue during a longer incubation period (Booth 2000). A large size with a small yolk reserve can be disadvantageous in an environment where food is scarce and difficult to locate (Booth 2006). A long and cool incubation can also lead to malformation and/or malfunction (Hare et al. 2008), and may have contributed to the deaths of some hatchlings in our study. But note that all surviving hatchlings are still alive 2 years later after outdoor rearing at Orokonui and have a normal growth rate (Mello et al. 2011).

In addition to the possible problems with soil moisture noted above, another source of egg incubation failure could have been our human-dug nests. In natural nests, eggs are sometimes contained in an air-filled chamber and expand during incubation to fill much of the unoccupied space (Thompson et al. 1996). The type of soil at Orokonui did not allow us to provide such an air-filled chamber to the eggs, and the only nest that survived was one that we opened twice to collect eggs for dissection. Perhaps those openings provided essential air flow; however, the situation is unclear because not all natural nests of tuatara contain an air-filled chamber. In some, eggs are completely encased in sandy soil but still expand during incubation and may hatch successfully (Thompson et al. 1996; NJN, pers. obs.), and the addition of sand to soil in our study should have enhanced drainage and gas exchange. Pintus et al. (2009) found that when eggs of green turtles (*Chelonia mydas*) were relocated to a new site, hatching success was reduced by 20% in comparison with in situ clutches. They also attributed the reduced hatching success to human-made nests. Another potential source of egg incubation failure could have been movements of the eggs, but early transportation (by air and by car from Stephens Island) seems an unlikely cause as most of the lab-incubated control eggs hatched. Sometimes egg incubation failure is attributed to clutch quality (i.e. some females produce eggs of lesser quality than others), but this effect was eliminated as we mixed all the clutches before incubation in the field and in the lab. Finally, bait for poisoning pest mammals was dropped at the ecosanctuary during incubation, but Ogilvie et al. (1997) have shown that significant contamination of water and soil is improbable after a single aerial application of brodifacoum baits.

Relocation of eggs proved difficult in practice, at least in tuatara. Relocation of eggs is a common strategy for conservation in turtles. Usually, in an attempt to improve survival, doomed eggs are relocated from vulnerable areas (subject to flooding or human disturbance) to a safer place (Kornaraki et al. 2006; Pfaller et al. 2009). These practices are well established for turtles and hatching success is usually high and comparable to that of in situ nests (Mrosovsky 2006). On the other hand, relocation of lizard eggs is rare and, when undertaken, is usually for research rather than conservation purposes. For example, Shine (2002) translocated eggs of

three-lined skinks (*Bassiana duperreyi*) to artificial nest sites, which were above the usual elevational limit of the species, to understand the evolution of viviparity in reptiles living in cold climates. At high altitude, hatching success was reduced to 20% and hatchling traits (body size, growth rate, running speed) were reduced.

Translocations of reproductive individuals and/or creation of artificial egg-laying sites (e.g. see Castilla & Swallow 1995) would be more successful, in terms of establishment of new reptile populations outside of their geographical range, than egg translocation alone.

Management implications

The tuatara lineage (including a sphenodontine not necessarily referable to *Sphenodon*) has clearly survived over recent epochs in climates that have been warmer (Miocene) and colder (Pleistocene) than that currently experienced by *S. punctatus* (Worthy & Grant-Mackie 2003; Jones et al. 2009). However, the surviving, relict populations of *Sphenodon punctatus* are likely to have reduced genetic diversity, and the rate at which temperatures are predicted to change over the coming century (IPCC 2007; Reisinger et al. 2010) is unprecedented, making adaptation problematic. An important component of a management plan for tuatara thus seems to involve translocation to other locations that are thermally suitable not only for adult organisms (in terms of thermal preference, tolerance, and physiological performances; see Besson & Cree 2010, 2011), but also for developing organisms (in terms of hatching success and sex ratio). Regions that are currently cooler than natural habitat may become increasingly important as future translocation sites.

Based on our data, and given that assessments of habitat in other respects (as per criteria in the Tuatara Recovery Plan; Gaze 2001) seem suitable, we suggest that translocation of tuatara to the Orokonui Ecosanctuary in southern New Zealand is appropriate to consider further. Although only female tuatara were produced during egg incubation at Orokonui, new suitable nesting sites have been created recently that are warm enough to produce male tuatara, provided that females lay their eggs early enough in the season so that the TSP occurs before the end of February, and that nests are sufficiently shallow. Shallow nesting may have been a general requirement for existence of tuatara in coastal Otago. On the other hand, tuatara that once existed further inland (e.g. at Alexandra and Wanaka; Fig. 1a) would have experienced much warmer soil temperatures over summer (similar to those on Stephens Island) and much cooler temperatures over winter at the same depths, enabling production of both sexes from deeper nests (Besson 2009).

Long-term monitoring of female nesting behaviour will be required at Orokonui to determine the viability of a translocated population. Philopatry is the primary determinant of nest site choice in female tuatara (Refsnider et al. 2010), so it will be important to study how translocated females colonise a new nesting area. The timing of oviposition and nest depth should also be investigated to determine if females respond to a new, cooler environment by nesting earlier or later in the season or by digging shallower nests to increase the proportion of male offspring. Doody et al. (2006) have shown that female nest site choice compensates for climatic differences (along a latitudinal gradient spanning 19°) among populations of Australian water dragons. Shallower nests usually experience higher mean temperatures and greater maximum daily temperatures, which in turn may enhance developmental rate and offspring fitness (Warner & Shine 2008). But, because warm nests are typically drier, eggs face a greater risk of desiccation. Therefore, nesting females must choose nesting sites that offer suitable hygric as well as thermal conditions (Warner & Andrews 2002). It is also worth noting that the trend observed here in biased sex ratio is likely to change in the context of climate change, as an increase of soil temperature by 3°C (IPCC 2007) would allow the production of males (and reduced incubation length) at the proposed site. In the meantime, induced egg-laying combined with artificial incubation of some eggs could be used as a precaution to ensure the production of both sexes. These techniques are well established for tuatara and have been used successfully as tools in the establishment of new populations (Thompson 1990; Daugherty 1998; Nelson et al. 2004c).

Acknowledgments

For providing the necessary permits, approval, and consultation, we are grateful to the Department of Conservation (permits # NM-18855-RES and NELCO-12497), the University of Otago Animal Ethics Committee, Ngāti Koata (kaitiaki or guardians of tuatara from Stephens Island/Takapourewa) and Ngāi Tahu (mana whenua of Otago). We are especially grateful to Peter Gaze for facilitating the study and to Orokonui Ecosanctuary personnel, especially Chris Baillie and Elton Smith, for their help and support. We thank Sue Keall and Jeanine Refsnider as well as numerous others for help during egg collection on Stephens Island and during preparation of the incubation site at Orokonui. We thank the University of Otago Department of Zoology for funding and technical assistance. We also thank Amy Armstrong and Kim Garrett for assistance with animal care, and the Cree Lab, David Booth and an anonymous reviewer for comments that improved the manuscript.

References

Armstrong DP, Seddon PJ 2008. Directions in reintroduction biology. Trends in Ecology and Evolution 23: 20–25.

Besson AA 2009. Effects of cool temperature on egg incubation, thermoregulation and physiological performance of tuatara (*Sphenodon punctatus*): implications for conservation programmes. Unpublished PhD thesis, University of Otago, Dunedin, New Zealand. 165 p.

Besson AA, Cree A 2010. A cold-adapted reptile becomes a more effective thermoregulator in a challenging environment. Oecologia 163: 571−581.

Besson AA, Cree A 2011. Integrating physiology into conservation: an approach to help guide translocations of a rare reptile in a warming environment. Animal Conservation 14: 28–37.

Booth DT 2000. Incubation of eggs of the Australian broad-shelled turtle, *Chelodina expansa* (Testudinata: Chelidae), at different temperatures: effects on pattern of oxygen consumption and hatchling morphology. Australian Journal of Zoology 48: 369−378.

Booth DT 2006. Influence of incubation temperature on hatchling phenotype in reptiles. Physiological and Biochemical Zoology 79: 274−281.

Bull JJ, Vogt RC, McCoy CJ 1982. Sex determining temperatures in turtles: a geographic comparison. Evolution 36: 326−332.

Castilla AM, Swallow JG 1995. Artificial egg-laying sites for lizards: a conservation strategy. Biological Conservation 72: 387−391.

Cree A, Butler D 1993. Tuatara recovery plan (*Sphenodon* spp.). Threatened Species Recovery Plan 9. Wellington, Department of Conservation. 71 p.

Cree A, Thompson MB, Guillette LJ Jr, Hay JM, McIntyre ME 1989. Embryonic development of tuatara in forested and open habitats on Stephens Island, New Zealand. New Zealand Journal of Zoology 16: 270 [abstract].

Cree A, Cockrem JF, Guillette LJ Jr 1992. Reproductive cycles of male and female tuatara (*Sphenodon punctatus*) on Stephens Island, New Zealand. Journal of Zoology London 226: 199−217.

Daugherty CH 1998. From dark days to a bright future: survival of the tuataras. ZooNooz April: 8−13.

Dendy A 1899. Outlines of the development of the tuatara, *Sphenodon* (Hatteria) *punctatus*. Quarterly Journal of Microscopical Sciences 42: 1−87.

Doody JS, Guarino E, Georges A, Corey B, Murray G, Ewert M 2006. Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination. Evolutionary Ecology 20: 307−330.

Dufaure JP, Hubert J 1961. Table de développement du lézard vivipare : *Lacerta (Zootoca) vivipara* Jacquin. Archives d'Anatomie Microscopique et de Morphologie Experimentale 50 : 309−328.

Ewert MA, Lang JW, Nelson CE 2005. Geographic variation in the pattern of temperature-dependent sex determination in the American snapping turtle (*Chelydra serpentina*). Journal of Zoology London 265: 81−95.

Gaze P 2001. Tuatara recovery plan 2001-2011. Threatened Species Recovery Plan 47. Wellington, Department of Conservation. 38 p.

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, Beggs K, Young JE, Doody JS 2005. Modelling development of reptile embryos under fluctuating temperature regimes. Physiological and Biochemical Zoology 78: 18−30.

Hamel J 2001. The archaeology of Otago. Wellington, Department of Conservation. 222 p.

Hare KM, Pledger S, Daugherty CH 2008. Low incubation temperatures negatively influence locomotor performance and behavior of the nocturnal lizard *Oligosoma suteri* (Lacertidae: Scincidae). Copeia 2008; 16−22.

IPCC 2007. Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change (IPCC). Cambridge University Press.

Jones MEH, Tennyson AJD, Worthy JP, Evans SE, Worthy TH 2009. A sphenodontine (Rhynchocephalia) from the Miocene of New Zealand and palaeobiogeography of the tuatara (*Sphenodon*). Proceedings of the Royal Society B 276: 1385−1390.

Kornaraki E, Matossian DA, Mazaris AD, Matsinos YG, Margaritoulis D 2006. Effectiveness of different conservation measures for loggerhead turtle (*Caretta caretta*) nests at Zakynthos Island, Greece. Biological Conservation 130: 324−330.

Lowe CB, Bejerano G, Salama SR, Haussler D 2010. Endangered species hold clues to human evolution. Journal of Heredity 101: 437–447.

Mello RSR, Fay V, Smith E, Hare KM, Cree A 2011. Adjustment of juvenile tuatara (*Sphenodon punctatus*) to a cool-climate ecosanctuary. In: Abstracts of papers presented at the 14th Biennial Conference of the Society for Research on Amphibians and Reptiles in New Zealand, Tautuku, Otago, New Zealand, 11–13 February 2011. New Zealand Journal of Zoology 38: 272–273.

Miller HC, Andrews-Cookson M, Daugherty CH 2007. Two patterns of variation among MHC Class I loci in tuatara (*Sphenodon punctatus*). Journal of Heredity 98: 666−677.

Mitchell NJ, Nelson NJ, Cree A, Pledger S, Keall SN Daugherty CH 2006. Support for a rare pattern of temperature-dependent sex determination in archaic reptiles: evidence from two species of tuatara (*Sphenodon*). Frontiers in Zoology 3: 9.

Mitchell NJ, Kearney MR, Nelson NJ, Porter WP 2008. Predicting the fate of a living fossil: how will global warming affect sex determination and hatching phenology in tuatara? Proceedings of the Royal Society B 275: 2185−2193.

Moffat LA 1985. Embryonic development and aspects of reproductive biology in the tuatara (*Sphenodon punctatus*). In: Gans C, Billet F, Maderson PFA eds Biology of Reptilia, development B. Vol. 14. New York, John Wiley. Pp. 495−521.

Mrosovsky N 1988. Pivotal temperatures for loggerhead turtles (*Caretta caretta)* from northern and southern nesting beaches. Canadian Journal of Zoology 66: 661−669.

Mrosovsky N 2006. Distorting gene pools by conservation: assessing the case of doomed turtle eggs. Environmental Management 38: 523−531.

Nelson NJ, Keall SN, Brown D, Daugherty CH 2002. Establishing a new wild population of tuatara (*Sphenodon guntheri*). Conservation Biology 16: 887–894.

Nelson NJ, Thompson MB, Pledger S, Keall SN, Daugherty CH 2004a. Do TSD, sex ratios, and nest characteristics influence the vulnerability of tuatara to global warming? International Congress Series 1275: 250−257

Nelson NJ, Cree A, Thompson MB, Keall SN, Daugherty CH 2004b. Temperature-dependent sex determination in tuatara. In: Valenzuela N, Lance V eds Temperature dependent sex determination in vertebrates. Smithsonian Books, Washington, DC. Pp. 53−58.

Nelson NJ, Thompson MB, Pledger S, Keall SN, Daugherty CH 2004c. Egg mass determines hatchling size, and incubation temperature influences post-hatching growth, of tuatara *Sphenodon punctatus*. Journal of Zoology London 263: 77−87.

Nelson NJ, Moore JA, Pillai S, Keall SN 2010. Thermosensitive period for sex determination in tuatara. Herpetological Conservation and Biology 5: 324−329.

Newman DG 1982. Tuataras, *Sphenodon punctatus,* and burrows, Stephens Island. In: Newman DG ed New Zealand herpetology. New Zealand Wildlife Service Occasional Publication No. 2. Pp. 213−221.

Newman DG, Watson PR, McFadden I 1994. Egg production by tuatara on Lady Alice and Stephens Island New Zealand. New Zealand Journal of Zoology 21: 387−398.

Ogilvie SC, Pierce RJ, Wright GRG, Booth LH, Eason CT 1997. Brodifacoum residue analysis in water, soil, invertebrates, and birds after rat eradication on Lady Alice Island. New Zealand Journal of Ecology 21: 195−197.

Olsson M, Shine R 1997. The seasonal timing of oviposition

in sand lizards (*Lacerta agilis*): why early clutches are better. Journal of Evolutionary Biology 10: 369−381.
Pfaller JB, Limpus CJ, Bjorndal KA 2009. Nest-site selection in individual loggerhead turtles and consequences for doomed-egg relocation. Conservation Biology 23: 72−80.
Pintus KJ, Godley BJ, Mcgowan A, Broderick AC 2009. Impact of clutch relocation on green turtle offspring. Journal of Wildlife Management 73: 1151−1157.
Refsnider JM, Keall SN, Daugherty CH, Nelson NJ 2009. Does nest guarding in female tuatara (*Sphenodon punctatus*) reduce nest destruction by conspecific females? Journal of Herpetology 43: 294−299.
Refsnider JM, Daugherty CH, Keall SN, Nelson NJ 2010. Nest-site choice and fidelity in tuatara on Stephens Island, New Zealand. Journal of Zoology 280: 396−402.
Reisinger A, Mullan B, Manning M, Wratt D, Nottage R 2010. Global and local climate change scenarios to support adaptation in New Zealand. In: Nottage AC, Wratt DS, Bornman JF, Jones K eds Climate Change Adaptation in New Zealand. New Zealand. Future scenarios and some sectoral perspectives. Wellington, Climate Change Centre, National Institute of Water and Atmospheric Research (NIWA. Pp. 26–43.
Sherley GH, Stringer IAN, Parrish GR 2010. Summary of native bat, reptile, amphibian and terrestrial invertebrate translocations in New Zealand. Science for Conservation 303. Wellington, Department of Conservation, 39 p.
Shine R 2002. Reconstructing an adaptationist scenario: what selective forces favor the evolution of viviparity in montane reptiles? The American Naturalist 160: 582−593.
Thompson MB 1990. Incubation of eggs of tuatara, *Sphenodon punctatus*. Journal of Zoology London 222: 303–318.
Thompson MB, Daugherty CH 1992. Living a lie: New Zealand's tuatara. Australian Natural History 23: 928–935.
Thompson MB, Packard GC, Packard, MJ, Rose B 1996. Analysis of nest environment of tuatara *Sphenodon punctatus*. Journal of Zoology London 238: 239–251.
Towns DR, Daugherty CH 1994. Patterns of range contractions and extinctions in the New Zealand herpetofauna following human colonisation. New Zealand Journal of Zoology 21: 325–339.
Towns DR, Ferreira SM 2001. Conservation of New Zealand lizards (Lacertilia: Scincidae) by translocation of small populations. Biological Conservation 98: 211–222.
Towns DR, Parrish GR, Tyrrell, CL, Ussher GT, Cree A, Newman DG, Whitaker AH, Westbrooke I 2007. Responses of tuatara (*Sphenodon punctatus*) to removal of introduced Pacific rats from islands. Conservation Biology 21: 1021–1031.
Warner DA, Andrews RM 2002. Nest-site selection in relation to temperature and moisture by the lizard *Sceloporus undulatus*. Herpetologica 58: 399−407.
Warner DA, Shine R 2008. Maternal nest-site choice in a lizard with temperature-dependent sex determination. Animal Behaviour 75: 861−870.
Worthy TH, Grant-Mackie JA 2003. Late-Pleistocene avifaunas from Cape Wanbrow, Otago, South Island, New Zealand. Journal of the Royal Society of New Zealand 33: 427−485.

Editorial Board member: Wayne Linklater
Received 14 March 2011; accepted 22 August 2011