SECURING THE FUTURE OF THREATENED TUATARA POPULATIONS WITH ARTIFICIAL INCUBATION

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Abstract.—Artificial incubation is a conservation technique used to provide founders for new or to augment existing reptile populations with minimal impact on the original population. It relies on the premise that hatching success of eggs in artificial conditions is high relative to natural nests. Our goal was to assist with the rescue of populations on the brink of extinction by incubating eggs produced by Tuatara from small islands inhabited by introduced rats. We incubated eggs produced over an 18 year period by Tuatara originating from Little Barrier, Cuvier, Stanley, and Red Mercury Islands, New Zealand, while they were in captivity awaiting return after rat eradications. The most successful results came from the Little Barrier stock where the population numbers increased dramatically, with eggs produced by all four mothers and high hatching success. Stanley Island stock produced the least successful outcome. Adults from Stanley Island suffered high mortality in captivity, and surviving females produced few eggs, with comparatively low hatching success. On balance, rescuing the genetic stock of remnant populations through captive incubation gives conservation programs time to deal with causes of decline and to plan for future success. However, supplementation in the future from other wild populations is likely to be necessary to ensure long term genetic variability and therefore viability of these populations, in particular Stanley Island.

Key Words.—captive incubation; hatching success; reptile; *Sphenodon*; Tuatara

INTRODUCTION

Conservation of threatened species may require techniques and actions that are controversial, due to the urgency with which they must occur, the absence of sufficient numbers with which to develop techniques via experimentation, and the paucity of information available at the time. For example, translocations have been used in attempts to restore species to former ranges under the assumption that more populations and increased habitat area will improve their conservation status, but subsequent monitoring indicates this technique does not necessarily contribute to improving the status of those species due to failure of many of these attempts to produce self-sustaining populations (Griffith et al. 1989; Wolf et al. 1998).

Incubation of eggs in artificial conditions to increase survival to hatching relative to natural nests is a conservation technique used to provide founders for new, or to augment existing, reptile populations with little risk to the original population (Nelson et al. 2002; Alberts et al. 2004). Subsequent head-starting of hatchlings can also improve recruitment by improving survival through vulnerable juvenile life stages (Alberts 2007). However, temperatures during incubation can affect appearance and performance traits that potentially affect fitness of resulting adults (Van Damme et al. 1992; Elphick and Shine 1998; Nelson et al. 2006). In addition, head-starting may allow survival of sub-optimal animals due to the special care they receive in captivity, many of which may not survive post-translocation (Chiszar et al.

1993). Planning experimental approaches, accurately recording events, and monitoring can allow us to learn from the application of conservation techniques for more informed conservation action in the future (Seddon et al. 2007), but information on threatened species often arises through long term associations with programs where information accumulates slowly. We report on the success of an 18-year program using captive incubation as a conservation tool for a threatened reptile.

Tuatara (*Sphenodon punctatus*) are medium-sized, long-lived reptiles that are the sole living representatives of the Order Rhynchocephalia (Hay et al. 2010). Tuatara survived only in New Zealand, where their natural range was reduced to 32 offshore islands due to predation by introduced mammals. Many of these islands were either small or were inhabited by introduced rats (*Rattus* sp.), or both, threatening the long term security of populations. (Cree and Butler 1993; Towns et al. 2007)

Surveys of Tuatara occupied islands were conducted by biologists from Victoria University of Wellington and the Department of Conservation, New Zealand, between 1988 and 1992. This lead to the discovery that four populations of Tuatara co-existing with Pacific Rats (*Rattus exulans*) were in decline (populations numbered only a few tens of individuals and no juvenile tuatara [< 180 mm snout-vent length] were seen; Gaze 2001). Between 1990 and 1992, all Tuatara that could be found on Little Barrier (n = 8), Cuvier (n = 6), Stanley (n = 15), and Red Mercury (n = 11) Islands were caught and removed to captive facilities for their own safe-keeping, pending

Table 1. Numbers of Tuatara captured from Pacific Rat-inhabited islands between 1990-1992, and facility where animals were held. All Tuatara captured were adults; no juveniles were observed.

Island	Tuatara captured (N)		Captive facility
	M	F	
Little Barrier	4	4	Little Barrier Island
Cuvier	4	2	Auckland Zoo
Stanley	8	7	Auckland/Hamilton Zoos
Red Mercury	2	9	Auckland Zoo

eradication of Pacific Rats from the islands (Barbara Blanchard pers. comm.; Table 1). Several captive facilities participated as no one facility was large enough to accommodate all animals captured from the four islands. Auckland and Hamilton Zoos provided facilities for animals from Cuvier, Stanley, and Red Mercury Islands, whereas Little Barrier Island Tuatara were kept in a purpose-built facility on the island. Tuatara in all facilities were housed in outdoor enclosures where they were protected from predators (Pers. Observ.).

Tuatara have a low reproductive rate, with females laying eggs once every two to nine years. In addition, clutch sizes are small compared with those of many other reptile species (Cree 1994; Mitchell et al. 2010). Hatching success in natural nests is about 48% (Thompson et al. 1996), and juvenile survival is low (Mitchell et al. in press). Our goal was to assist with the rescue of Tuatara populations on the brink of extinction by incubating eggs produced by Tuatara from Little Barrier, Cuvier, Stanley, and Red Mercury Islands and raising healthy juveniles to enter head-start programs that would prepare them for release.
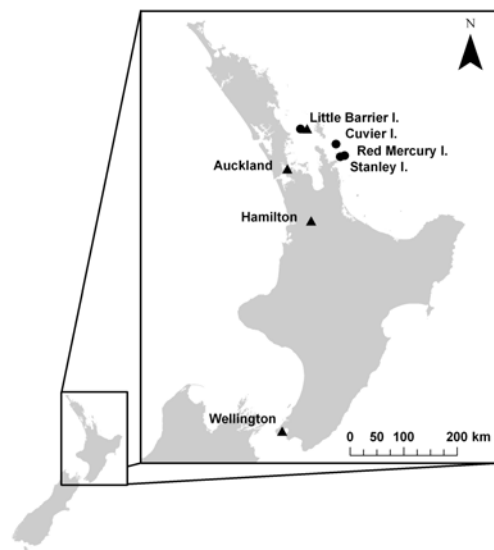

FIGURE 1. North Island of New Zealand showing Little Barrier, Cuvier, Stanley, and Red Mercury Islands (circles), and locations of captive facilities for Tuataras (triangles).

MATERIALS AND METHODS

We incubated eggs produced over an 18-year period by captive Tuatara originating from Little Barrier, Cuvier, Stanley, and Red Mercury Islands (Fig. 1). Eggs laid naturally or artificially induced from females (Nelson et al. 2004a) at the captive facilities were incubated at Victoria University of Wellington (VUW). All eggs were assigned a unique number written on the top surface of the egg with a soft (4B) graphite pencil, and the orientation of the egg was maintained throughout the study (Thompson 1990). Eggs were weighed to accuracy of 1 mg on a Sartørius (GMBH Type 1475, Gottengen, Germany) top pan balance on the day they were received at VUW, and randomly assigned to an incubation box. Incubation was conducted following the methods of Nelson et al. (2004a). Briefly, each incubation box comprised a sealed 2 liter plastic container half-filled with vermiculite moistened with distilled water (about.-170 kPa). Incubation temperatures were chosen to achieve the desired sex ratios of hatchlings, given that Tuatara sex is determined by temperature (Type 1b, males produced from incubation temperatures at or above 22° C; Cree et al. 1995; Nelson et al. 2004b). Early incubation regimes were constant 18° C, 21° C, 22° C, and an 18–23° C variable regime. However, once laparoscopy (Nelson 2002) confirmed the sex of hatchlings, eggs were incubated at 20° C (females) and 23° C (males) to obtain an even sex ratio. Eggs were weighed weekly, and water potential was maintained by adding distilled water to incubation boxes to compensate for small losses from the container and uptake by the eggs, as described by Thompson (1990).

Hatchlings were weighed, measured (snout-vent length (SVL)), and individually marked with a toe-clip within three days of hatching, then weighed and measured weekly. Hatchlings were initially raised indoors at VUW in colonial conditions in terraria furnished with a soil and leaf litter base, tree bark for shelter and a water dish. Food comprised naturally occurring invertebrates in leaf litter, and wild-caught Slaters (*Porcellio scaber*) and moths, supplemented weekly with captive-bred invertebrates (house flies, blow flies, or crickets). Enclosures were exposed to daily temperature variations between approximately 18–22° C and a 12:12 light/dark cycle beginning at 0600 h throughout the year (Duro-test® True-lite® power twist fluorescent tubes, Philadelphia, Pennsylvania, U.S.A., placed 750 mm above the enclosure).

Juveniles were returned to their source captive facility once they were feeding and appeared healthy. Typically, age at transfer ranged from one week to six months, although the first cohort to be incubated at 23° C was returned to its captive facility on Little Barrier Island at 11 months old, once the juveniles had attained a size suitable for laparoscopy to confirm sex (Nelson 2002).

TABLE 2. Years that clutches of eggs from (A) Little Barrier Island and (B) Cuvier Island were incubated at Victoria University of Wellington (VUW), showing number of eggs hatched/number of eggs received. Only eggs that arrived at VUW contributed to data on hatching success.

A)

Mother	1992	1994	1995	1997	1998	1999	2000	2001	2002	2003	2005	2006	Total (%)
Mrs O	0/4[1]				4/7		7/9[2]				4/7		55.6
Greta		10/10			6/7		7/9		9/14				80.0
Whero			9/10	14/15	0/7	3/5	11/12			14/14		9/10	82.2
Kowhai			0/9	12/13		2/5		5/11		7/10		3/11	49.2

B)

Mother	1995	1997	1999	2001	2003	2004	2007	Total (%)
RS0035	6/17[1]	0/15[1]	1/2[3]		0/20	15/19		30.1
RS0047		1/13[1]	11/12	0/13	3/14		3/7	30.5

[1] Clutch laid by induction
[2] An additional 5 eggs in clutch not sent to VUW because they had obvious signs of having perished, i.e. collapsed shell, moldy, discoloured.
[3] An additional 16 eggs in clutch not sent to VUW because they had obvious signs of having perished, i.e. collapsed shell, mouldy, discoloured.

We used an analysis of variance (SPSS 16.0, Statistical Package for the Social Sciences, Inc., Chicago, Illinois, USA) to investigate whether hatching success varied by island. We included all clutches produced by adults sourced directly from islands, but we excluded clutches resulting from artificially incubated Little Barrier Island stock (n = 3). Five eggs from one Little Barrier clutch and 16 eggs from one Cuvier clutch were also excluded as they had perished at the captive facility prior to eggs being sent to VUW. We acknowledge over-representation and multiple clutches by some mothers in the data from Little Barrier, but we could not analyze data in further detail due to small numbers of repeat clutches from females from Stanley and Red Mercury Islands. All data were normal and variances were equal. For all tests, α = 0.05

RESULTS

Eggs were naturally laid in enclosures between 4 November and 15 January (12–24 November, Cuvier; 26 November-15 January, Stanley; and 4 November - 4 January, Little Barrier; the only Red Mercury clutch laid naturally occurred on November 16). On three occasions, eggs were laid on the soil surface rather than in properly constructed nests, resulting in dehydration. Induction of eggs resulted in production of soft, poorly calcified eggs in six of 16 instances. All three instances involving induction of eggs from Cuvier Island stock resulted in poorly calcified eggs; two of these were conducted earlier than eggs were naturally laid by females from this island. The only instance of induction causing poor calcification of Stanley Island eggs also occurred prior to the usual laying time. However, induction of three females from Red Mercury Island occurred at similar times as those resulting in poor calcification from Cuvier and Stanley Islands (mid-October), and none of the Red Mercury eggs exhibited poor calcification. Induction ceased to be used as a technique for obtaining eggs

from these populations in 2002. Poorly calcified eggs and those laid on the soil surface did not hatch.

A total of 553 eggs from Little Barrier, Cuvier, Stanley and Red Mercury Islands were incubated between 1990 and 2007. Of these, 241 eggs hatched; an overall hatching success rate of 43.6%. The Little Barrier colony was the most productive, with 253 eggs incubated from 23 clutches and 152 eggs hatched (success rate = 60.1%). Red Mercury Tuatara produced 79 eggs in nine clutches, of which 35 hatched (44.3%). The Cuvier Island colony produced 132 eggs in 10 clutches, of which 40 eggs hatched (30.3%). Only 14 of 89 eggs in 10 clutches from Stanley animals hatched (15.7%). Above percentages refer to hatchlings/egg incubated from each island (percentages in following paragraphs are base on the mean hatchlings per clutch on each island).

All four females from Little Barrier produced eggs. The first clutch to hatch successfully was in 1994, two years after the program began. Hatching success ranged from 0–100% among clutches (average 63%; Table 2; Fig. 2). All Little Barrier females were capable of producing clutches every second year, and one female produced eggs four years in a row, although only three of those clutches resulted in hatchlings. Clutches from Little Barrier Tuatara had the highest hatching success of the four populations (Fig. 2). Three females resulting from the artificial incubation program (F1) also produced a clutch (potential F2) each as 9–11 year olds, but these eggs did not hatch. All adult Tuatara from Little Barrier Island survived throughout the captive program.

The two females removed from Cuvier Island began producing eggs in 1995, five years after they were brought into captivity and were capable of producing eggs at least biannually (Table 2). Hatching success ranged from 0–92% among clutches (mean 33%; Fig. 2). All adults taken into captivity from Cuvier Island survived throughout the captive program.

Eggs hatched successfully from four of the seven Stanley Island females. Hatching success of Stanley
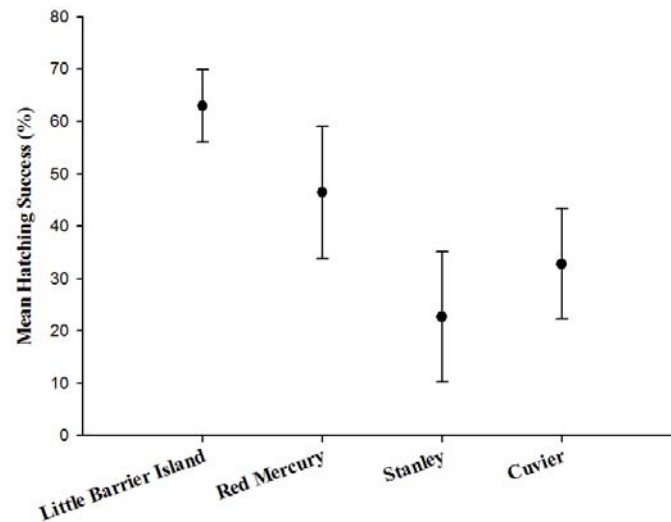
FIGURE 2. Mean percentage hatching success (± 1 SE) for Tuatara clutches under captive conditions separated by island group of origin. numbers in parentheses indicate number of clutches.

Island clutches (mean 23%) was significantly less than Little Barrier Island clutches ($F_{4,50}$ = 23.63; P < 0.001; Fig. 2). The first successful clutch from Stanley Island was not produced until 1998, eight years after the arrival of adults into captivity. Six adult Tuatara from Stanley Island died at the captive facility between 1990 and 2001, including three females (Barbara Blanchard pers. comm.).

The mean hatching success of Red Mercury Island clutches was 46% (Fig. 2). Adults were returned to Red Mercury Island relatively early on in the incubation program following successful eradication of rats. Two males and eight females were released in 1996, and one female in 1998, six and eight years, respectively, after capture. As a result, captive incubation ceased for eggs from this island in 1998. Five of the nine Red Mercury Island females laid successful clutches. Clutches from Stanley and Red Mercury females were sporadic, providing no comparable information on relative success of clutches by individuals.

DISCUSSION

Four populations of Tuatara on the brink of extinction were rescued using a combination of actions: bringing survivors into captivity, captive incubation of eggs, head-starting of juveniles, eradication of introduced predators, and repatriation of survivors and offspring to their source island. We evaluated whether captive incubation could be used as a key element in this process, as the technique has been shown to increase the hatching success of Tuatara eggs from 48% in nature (Thompson et al. 1996) to 80–90% when eggs from healthy wild populations are used (Nelson et al. 2004a).

During the 18 years of this program, survival of adults, number of eggs laid, and hatching success varied among the four island captive colonies. The most successful results came from the Little Barrier stock where the population numbers increased dramatically, with eggs produced by all four females and high hatching success. Stock from Stanley Island were least successful. Adults from Stanley Island suffered high mortality in captivity, and surviving females produced few eggs. Hatching success of artificially incubated Stanley Island eggs in protected and stable environmental conditions, where they experienced no nest disturbance, was half that expected of wild nests on Stephens Island, where conspecifics destroy nests (Refsnider et al. 2009) and variable environmental conditions cause high mortality (Thompson et al. 1996).

A number of factors may have contributed to the variation in results. In particular, age and reproductive status of adults upon capture were not known. On Stephens Island, the densest and most abundant Tuatara population, 5.6% of female Tuatara were observed to have no vitellogenic follicles and were presumed to be reproductively inactive; regressed ovaries were also observed in a few old females suggesting Tuatara can outlive their reproductively active years (Cree et al. 1991). Stanley Island Tuatara may have been very old and reaching the end of their reproductive life span. In addition, competition from Pacific Rats prior to capture may have negatively influenced body condition and therefore reproductive output, especially in females (Tyrrell et al. 2000). These surviving Tuatara were at extremely low densities and widespread on the four islands, and Tuatara in a well-studied population on Stephens Island are known to have high home range fidelity

(Moore et al. 2009). Consequently the encounter rates of these survivors with each other in the years or decades prior to capture could have been very low or non-existent, effectively curtailing breeding. We are unsure how the absence of any interaction with conspecifics and breeding activity in years prior to captivity may affect resumption of breeding.

All adult captive colonies were under the care of either zoo or Department of Conservation staff, at three different facilities. Levels of experience and expertise among staff varied. Ability of staff to recognize nesting behavior of female Tuatara and subsequently locate nests was an important factor in retrieval of naturally laid eggs, determining number of nests found and quality of eggs if discovery of nests was delayed. Egg quality could also have been affected by timing of inductions; timing of natural egg laying in these northern populations of Tuatara was uncertain (Tyrrell et al. 2000). Premature induction would produce eggs with incomplete calcification of shells. However, our results are inconclusive on this aspect, as Red Mercury Island eggs induced at similarly early times to Stanley Island eggs were adequately calcified for successful incubation, and these two islands are adjacent.

As a result of artificial incubation, three of the study populations are substantially larger. The population on Little Barrier Island has now grown from eight to over 150 individuals (Moore et al. 2008; Barbara Blanchard pers. comm.). The Red Mercury Island population has grown from the original 11 adults captured to 25, all of which were released on to Red Mercury Island by 2001. Forty Cuvier Island hatchlings increased that population from six to 46. However, only 14 juveniles were produced from Stanley Island eggs and 6 of the original adults (3 males and 3 females) have died in captivity (causes largely unknown) resulting in a population increase of 53% (from 15 to 23 individuals; Barbara Blanchard pers. comm.). Infrequent breeding and K-selected life-history characteristics are features of New Zealand threatened species (Cree 1994; Elliott et al. 2006). However, even the critically endangered New Zealand parrot, the Kakapo (*Strigops habroptilus*), with poor hatching success and an apparent reliance on infrequent mass-fruiting events of Rimu Trees (*Dacrydium cupressinum*) for breeding, has had more production than resulted from Tuatara on Stanley Island. The numbers of kakapo rose by 69% between 1995 and 2002 (Elliott et al. 2006).

Hatching success is just the first stage in assessing the long-term value of artificial incubation. Hatchlings produced need to survive, grow and reproduce to ultimately contribute to population growth. Holding hatchlings in captivity until islands are ready for repatriation allows monitoring of the offspring. Eradication of rats required more than a decade to organize for Little Barrier Island (Ombler 2004). In that time, three of the hatchling females produced by artificial incubation (F1) reached

reproductive age (9–11 years) and successfully produced eggs (potentially F2) in captivity, providing early signs that artificially incubated offspring are healthy. No hatchlings resulted from these eggs, but we are uncertain whether this is a result of no males in the enclosure (due to inconclusive laparoscopies of a few individuals), or because males held with these females were not yet sexually mature. Artificially incubated young from Stephens Island have reached sexual maturity and successfully produced viable eggs in captive conditions (authors, unpubl. data), so we suspect the Little Barrier eggs were not fertilized rather than there being a problem with embryonic development in these instances. Information on survival to sexual maturity of hatchlings resulting from artificially incubated eggs is not available for the other island stocks involved in this study.

Artificial incubation has been used with varying success for all groups of reptiles, and artificially incubated young have been used as founders for translocations with varying success (e.g. Nelson et al. 2002; Bell et al. 2005). Translocations using stock originating in captivity are likely to be less successful than when animals are taken directly from the wild (e.g. Wolf et al. 1998), as captive breeding may result in loss of natural behaviors and genetic variation. Captive breeding may also affect performance and morphology; for example, size and sprint speed vary between captive and wild caught critically-endangered Otago Skinks (*Oligosoma otagense*; Connolly and Cree 2008). The offspring in this study result from parents of wild origin held in captivity for a relatively short period (with respect to their average life span; Mitchell et al. 2010), not as a result of a long term captive breeding program where behavioral and genetic changes in particular may reduce fitness under wild conditions. Loss of genetic variation within the population may have been modest, as reductions in population numbers due to invasive rats occurred only recently and over a short time frame (i.e., only one or a few generations at Little Barrier Island; Moore et al. 2009). However, success of artificially incubated and raised young will only be confirmed once they are surviving and successfully breeding back on their islands.

On balance, artificial incubation is a useful rescue technique, even if females take time to settle into egg production in captivity, lay clutches infrequently and are nearing the end of their reproductive life. In the short to medium term, numbers can be increased for very small and threatened populations. In fact, for the Stanley Island population, artificial incubation occurred just in time to secure the genetic material of the last remaining Tuatara on that island. Retaining the genetic stock of remnant populations through captive incubation gives conservation programs time to deal with causes of decline and to plan for future success. Long term success of these populations is uncertain due to the extended time juveniles take to reach sexual maturity and high mortality of juveniles

in the wild (Mitchell et al. 2010). In addition, some populations will likely depend on supplementation of animals from other populations to restore genetic diversity if they are to survive (IUCN 1987; Jamieson et al. 2006; Moore et al. 2008).

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