Attaching radio transmitters does not affect mass, growth, or dispersal of translocated juvenile tuatara

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The IUCN guidelines for conservation translocation (IUCN/SSC 2013) emphasize the importance of post-release monitoring. Such monitoring provides a feedback mechanism to update and enhance current protocols for translocations (Armstrong and Seddon 2008). Monitoring using radio telemetry can be a valuable method for accruing post-release information, including estimates of survival, habitat use and dispersal distance (Le Gouar et al. 2012). Telemetry is an effective technique for studying lepidosaurs (tuatara, lizards, and snakes), many of which are cryptic and/or have secretive behaviors that make them otherwise difficult to detect. An important assumption of telemetry-derived data is that tagged individuals are not adversely affected by the attached devices (White and Garrott 1990), yet many studies do not specifically address the effects of radio-tagging on animals (Millsbaugh and Marzluff 2001). The Tuatara (Sphenodon punctatus), an endemic reptile of New Zealand, has been reintroduced to Orokonui Ecosanctuary (hereafter Orokonui; Te Korowai o Mihiwaka; 45.766°S, 170.6°E) in the southeastern South Island of the country. In total 87 animals, including 57 juveniles, have been translocated. Tuatara are diurno-nocturnal, burrowing reptiles with low detection probabilities (Cassey and Ussher 1999), particularly for juveniles (Dawbin 1982). We used radio telemetry to monitor dispersal distance and habitat use of juveniles during the establishment phase (IUCN/SSC 2013). Telemetry has been employed for monitoring adult Tuatara, but has rarely been used on juveniles (Table 1). A pilot study was first performed to try several attachment methods. We then compared the performance of tagged versus untagged juveniles that were free-released into the ecosanctuary. Specifically, we evaluated whether body mass, growth rates, and dispersal behaviors differed between tagged and untagged juveniles over five months following release.

**Pilot study methods.**—In the pilot study we used dummy transmitters (ovoid beads, clay molds, or non-functional transmitters) that were approximately the same size (23 mm × 12 mm × 7 mm, LWH) and mass (3.9 g) as the functional transmitters (PD-2T, Holohil Systems, Carp, Ontario, Canada, with a 20-cm long whip antenna on the posterior end) used for the free-release of juveniles. Dummy and functional transmitters had cylindrical tubes for the passage of harness material. The juveniles tagged in the pilot study had a mean mass of 93 g (range = 87–107 g), and thus transmitters and harness were < 4.3% body weight. We compared four tag attachment methods on juveniles resident in outdoor enclosures (ca. 2 m × 1 m × 0.8 m, LWH) at Orokonui. Each enclosure had a soil substrate, rocks and logs for cover, and artificial burrows made of drainage pipe (Novoflo™) (Mello et al. 2013).

The first method was a “backpack harness” modified from Ussher (1999). Straps made of 6-mm flat elastic were threaded through the cylindrical attachment tubes, then wrapped around the forelimbs in a “figure 8” manner so as to allow the transmitter to sit slightly to one side of the dorsal surface of the animal, just posterior to the neck. Simple stitches (using “Strong Thread,” Sullivan’s International®) were made to join the elastic straps together where they crossed on the flanks of the animal. The second method (“surgical-tape”) was a variation on the adhesive tape method, instead using duct-tape (Leukopor micropore®; 25 mm wide) that was wrapped around the tail twice to secure the transmitter at the base of the tail, just distal to the cloaca, such that the transmitter sat on the side of the tail leaving the antenna to run alongside it. This method has been used for monitoring New Zealand lizards (e.g., Oligosoma otagense) (Germano 2007). The third method (“duct-tape”) was a variation on the adhesive tape method, instead using duct-tape (initially 3M®, 24 mm wide) that was used and wrapped only once around the tail. Previous research on reptiles has found that duct-tape lasts longer than other types of tape; however, it may cause superficial scars (Wylie et al. 2011). The fourth method was a pelvic harness (hereafter “pelvic attachment”) modified from a design used on Great Basin Collared Lizards (Crotaphytus bicinctores: J Cossell, pers. comm.). This method involved placing the transmitter on the dorsal surface of the pelvic girdle, where it was secured with round elastic threaded through the tubes then criss-crossed under the ventral surface such that the cloacal vent was not obstructed. Because Tuatara have a gap in the spines dorsal to the pelvis, the transmitter sat securely on the animals and was held firmly in place with a square knot. The backpack harness, surgical-tape, and duct-tape methods were initially tested on one juvenile each for approximately 35 days and the pelvic attachment

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The method was subsequently tested on two juveniles for 20 days; the pelvic attachment was trialled for a shorter duration due to time constraints.

Pilot study results.—Results from the pilot study showed that the backpack harness remained securely attached; however, it caused minor swelling of the animal’s shoulder, which had subsided one month after the harness was removed. By contrast, another juvenile shed the tag that was attached with surgical tape in < 30 days. Initially, the duct-tape method remained attached for < 4 days, but after changing to a more flexible, non-water or UV-resistant tape (Duct-tape II) and wrapping the tape around the tail twice, the attachment stayed on for the remainder of the pilot study (33 days). No injury resulted from the tape methods, and any tape residue was wiped clean with ethanol. Subsequently, the pelvic harness was trialled as we felt that it might offer advantages for Tuatara, including having a more symmetrical placement of the transmitter (better for weight distribution) and possibly less restriction of burrowing; however, juveniles escaped from this attachment in < 4 days. The pilot study informed our decision to use the backpack harness to monitor the translocation of juveniles because of the increased likelihood it would remain attached for the five months of the study, including during molting. We refined this method for the free-release (main study) to try and reduce the possibility of harness-related injury by: 1) using a 3-mm flat elastic (overlocked to reduce elasticity) to permit a better and slightly looser fit of the harness on the juveniles; 2) using a polyester nylon loop threaded through the cylindrical attachment tubes and finished with a square knot to allow elastic straps to be more evenly distributed on the animal; and 3) an additional simple stitch connecting the elastic straps where they crossed on the ventral surface to reduce abrasion by reducing movement of the harness attachment (Fig. 1). Furthermore, we recaptured most tagged juvenile Tuatara at approximately 50-day intervals to check for adverse effects from the harness fit (e.g., skin abrasion and/or swelling).

Main study methods.—The vegetation at the study site for the free-release of juvenile Tuatara comprised mostly regenerating native Kānuka (Kunzea ericoides) forest, with remnant patches of podocarp-broadleaved forest (Coprosma spp., Lemonwood [Pittosporum eugenioides], Mahoe [Melicytus ramiflorus], Mapau [Myrsine australis], and modified bush-margins of bracken (Pteridium spp.). Juveniles were released in the austral spring, a period of increasing temperature and activity for Tuatara. A total of 14 wild-caught juveniles from Stephens Island (Takapourewa) were released on 17 October 2012. An additional 41 captive-reared juveniles were released on 7 November 2012: 28 from Nga Manu Nature Reserve (hereafter Nga Manu) and 13 from Orokonui. Two more juveniles from Orokonui were released into the same area on 3 December 2012, following completion of additional disease testing. Prior to the free-release of tagged and untagged juveniles, a passive integrated transponder (PIT) tag (11 mm × 2 mm; AVID Identification Systems, Inc., Norco, California, USA) was inserted subcutaneously just anterior to the left rear leg for individual identification. We took several morphometric measurements of juveniles at release and recapture: snout-to-vent length (SVL); vent-to-tail-length; length of the regenerated portion of the tail; and mass. The mass of all juveniles at release ranged from 44–177 g (mean = 93.7 g) and SVL at release ranged from 106–169 mm (mean = 138.9 mm). We attached transmitters onto subsets of the three groups of juveniles: 10 wild-sourced,
Table 1. History of telemetry use with Tuatara (*Sphenodon punctatus*) (updated from Ussher 1999).

<table>
<thead>
<tr>
<th>Adult or juvenile</th>
<th>Attachment</th>
<th>Resident or translocation</th>
<th>Sample size (N)</th>
<th>Period of attachment</th>
<th>Data collected</th>
<th>Negative effects on animals</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>Surgical tape</td>
<td>Resident (Lady Alice Island)</td>
<td>6</td>
<td>≤ 9 days</td>
<td>Body temperature</td>
<td>Not observed</td>
<td>Tyrrell 2000</td>
</tr>
<tr>
<td>Adult</td>
<td>Hind-quarter mount</td>
<td>Resident (Stephens Island)</td>
<td>≤ 4 per trip for 3 trips</td>
<td>≤ 9 days</td>
<td>Body temperature</td>
<td>No data</td>
<td>Barwick 1982</td>
</tr>
<tr>
<td>Adult</td>
<td>Backpack</td>
<td>Resident (Stephens Island)</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>Overloaded animals; entrapment in burrows</td>
<td>Newman 1980</td>
</tr>
<tr>
<td>Adult</td>
<td>Backpack</td>
<td>Resident (Stephens Island)</td>
<td>10</td>
<td>≤ 12 months</td>
<td>Habitat use, behavior and body condition</td>
<td>Occasional swollen limbs</td>
<td>L. Anderson, pers. comm., 2013</td>
</tr>
<tr>
<td>Adult</td>
<td>Backpack</td>
<td>Resident (Taranga Island)</td>
<td>10</td>
<td>ca. 1 month</td>
<td>Nesting habits</td>
<td>Not observed</td>
<td>J. Moore, pers. comm., 2013</td>
</tr>
<tr>
<td>Adult</td>
<td>Backpack</td>
<td>Translocation (Titi Island)</td>
<td>7</td>
<td>ca. 2 months</td>
<td>Dispersal and movements</td>
<td>Not observed</td>
<td>Nelson 1998; N. Nelson, pers. comm., 2013</td>
</tr>
<tr>
<td>Adult</td>
<td>Backpack</td>
<td>Translocation (Zealandia™, Wellington)</td>
<td>16</td>
<td>≤ 12 months</td>
<td>Survival, dispersal, and growth</td>
<td>Greater dispersal distances than untagged conspecifics</td>
<td>McKenzie 2007</td>
</tr>
<tr>
<td>Adult</td>
<td>Backpack</td>
<td>Translocation (Motohoua Island)</td>
<td>29</td>
<td>≤ 16 months</td>
<td>Dispersal and habitat use</td>
<td>Frequent damage to scales; occasional swollen limbs and broken skin</td>
<td>Ussher 1999</td>
</tr>
<tr>
<td>Adult</td>
<td>Backpack</td>
<td>Translocation (Orokonui Ecosanctuary)</td>
<td>10</td>
<td>≤ 4 months</td>
<td>Nesting behavior, body temperature, and dispersal</td>
<td>Occasional damage to scales; occasional swollen limbs and broken skin</td>
<td>A. Besson and S. Adolph, pers. comm., 2013</td>
</tr>
<tr>
<td>Adult</td>
<td>Backpack</td>
<td>Translocation (Tiritiri Matangi)</td>
<td>9</td>
<td>&lt; 3 months</td>
<td>Dispersal and habitat use</td>
<td>Occasional swollen limbs</td>
<td>Ruffell 2005</td>
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<tr>
<td>Juvenile</td>
<td>Intraperitoneal implant</td>
<td>Resident (Stephens Island)</td>
<td>4</td>
<td>&lt; 1 month</td>
<td>Movement patterns and habitat use</td>
<td>No data</td>
<td>M. McIntyre, pers. comm., 2013</td>
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<tr>
<td>Juvenile</td>
<td>Backpack</td>
<td>Translocation (Titi Islands)</td>
<td>2</td>
<td>ca. 2 months</td>
<td>Dispersal and movements</td>
<td>Not observed</td>
<td>Nelson 1998; N. Nelson, pers. comm., 2013</td>
</tr>
</tbody>
</table>
10 head-started at Nga Manu, and six head-started at Orokonui. We attempted to match the body size, sex composition (where known), and numbers of individuals in groups as closely as possible. On average, the total package (transmitter and harness) weighed 3.7% of the mass of the released juveniles (range = 2.6–5.4%). All measurements were taken by one author (SJ).

All 26 tagged juveniles were radio-tracked with TR-4 receivers (Telonics, Mesa, Arizona, USA) and hand-held 3-element Yagi folding directional antennas (Sirtrack Electronics, Havelock North, New Zealand) two times per week to minimize disturbance. We removed all transmitters after five months. Untagged juveniles were recaptured opportunistically throughout the duration of the study; only two of the untagged individuals were not recaptured. In addition, we recorded the distance and compass direction of all Tuatara movements. We measured distance (to the nearest cm) using a tape measure and compass direction from the previous sampling point or the release burrow. Distance and direction data were converted to GPS coordinates, relative to several fixed locations at the study site that were established using GNSS technology (Trimble R7 GNSS with Trimble Zephyr™ Geodetic 2 Antenna mounted on a range pole, Sunnyvale, California, USA, with < 3 cm accuracy). GPS coordinates for each animal were then used to calculate post-release dispersal defined as the straight-line distance between the initial release site and final recapture location for Tuatara (measured in ArcGIS 10.1; ESRI, Redlands, California, USA).

Statistical analysis.—We used analysis of variance (ANOVA) to test for effects of transmitters and animal source (i.e., Orokonui, Nga Manu, or Stephens Island) on proportional growth in snout–vent length (mm/mm initial SVL/day), proportional change in body mass (mg/mg initial body mass/day) and proportional dispersal distance (mm/mm initial SVL/day). Data were analyzed using the statistical program "R" (version 3.0.1; R Development Core Team 2013). Statistical significance was assumed at $p < 0.05$. All data satisfied the assumptions of the statistical tests used.

Main study results.—We monitored transmitters attached to 26 reintroduced juveniles for a cumulative total of 3750 animal days (range = 71–158 days per animal). Three juveniles (11.5%) escaped from their harnesses during our study; however, all of these animals were recaptured and had their harnesses replaced within 10 days. There were also three tagged animals (11.5%) recaptured that exhibited skin abrasion and/or swelling of one shoulder and arm; transmitters were removed when this was detected. Harness-induced injury was first noticed ca. four months after release. One tagged individual was found dead at ca. three months post-release, probably as a result of a human-related crush injury that did not occur while radio tracking. Our initial analysis of tagged versus untagged individuals showed that including source population as a factor was not significant in any analysis ($P > 0.05$). Subsequently, we ran our analyses omitting source population; the changes in proportional body mass ($F_{1,53} = 0.252, P = 0.618$), proportional growth rates ($F_{1,53} = 1.027, P = 0.316$), and dispersal distance ($F_{1,53} = 0.108, P = 0.744$) were not significantly different between tagged and untagged juveniles (Fig. 2).

Discussion.—Although radio-telemetry studies require the fundamental assumption that the use of radio tagging does not have a detrimental impact on tagged animals (White and Garrott 1990), many studies, particularly for reptiles, do not quantitatively test this assumption. In our study of reintroduced juvenile Tuatara we used animals that had no transmitters attached as controls. Our results showed that, over the five months post-release, tagging juvenile Tuatara did not negatively impact performance parameters, such as growth, body mass, or dispersal distance. A previous reintroduction of Tuatara to a different site, however, suggested that tagged adults dispersed further than untagged conspecifics, presumably due to disturbance from intensive (daily) monitoring (McKenzie 2007). Dispersal distance, in particular, is a critical issue in reintroduction biology (Armstrong and Seddon 2008), as populations can fail to establish if too many animals disperse far from release areas and are unable to contribute to the breeding population (Le Gouar et al. 2012). The reduced frequency of our monitoring regime, as a result of findings from this earlier study, might have lessened the negative impact of intensive radio-tracking. This illustrates the importance of disseminating post-release data in translocation projects, because while monitoring is important to understand the process of establishment and to improve future programs (Armstrong and Seddon 2008), it should not influence the behavior of released animals.

Our modified backpack harness had no apparent effect on the behavior of juveniles. For instance, we observed tagged juveniles exhibiting the same behaviors as untagged individuals, including foraging and sun-basking. However, one aspect of the welfare of tagged animals that is important to monitor is the presence of harness-induced injury (Millspaugh and Marzluff 2001). In our study three juveniles had skin abrasion and/or swelling of the shoulder and arm; two of these tagged animals had previously escaped their transmitters. It is probable that, when we reattached the harness, the elastic straps were tightened more firmly to prevent future harness loss. This highlights a fine line between having the attachment method too loose, too firm, or just right. Research on lizards has identified similar problems with animals either escaping from the backpack harness (e.g., Fisher and Muth 1995; Price-Rees and Shine 2011) or being injured by this attachment method (e.g., Goodman et al. 2009) despite the duration of monitoring usually being shorter. It should be noted that all of the injured juvenile Tuatara have been recaptured since the transmitters were removed, with the swelling having subsided. Thus, correct fitting and regular visual inspections of animals for abrasions or skin irritations and/or recaptures of tagged animals are important to reduce injuries during the long-term deployment of transmitters, especially when working with growing juveniles.

In summary, we encourage more telemetry studies of reptiles to use comparative data of tagged and untagged animals to evaluate the effects of monitoring, an admittedly difficult task, especially for juveniles due to their cryptic nature and secretive behaviors (Pike et al. 2008). Nevertheless, erroneous conclusions might be reached about the species’ ecology, life history, and behavior if tagged animals are not representative of the entire population (White and Garrott 1990; Millspaugh and Marzluff 2001).

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Extracting and Amplifying DNA from Skin Swabs of a Forest Dwelling Tree Frog Species (*Nyctixalus pictus*)

A major contributor to amphibian declines in tropical regions of southeastern Asia is deforestation (Bickford et al. 2010). In Singapore, it is estimated that within the last 200 years, up to 95% of original forest cover has been cleared (Brooks et al. 2003). Despite deforestation, anuran diversity in Singapore is high with almost 30 native species (Baker and Lim 2012) and at least two introduced species (Leong and Lim 2011). Given these high levels of diversity and a recent history of deforestation, Singapore is ideal for genetic investigations of fragmentation effects; however, for forest dwelling species which often have small or dwindling population sizes (Bickford et al. 2010), invasive sampling (i.e., muscle, liver, or toe-clipping) may be counterproductive to conservation management efforts. Thus, we decided to explore the feasibility of using minimally invasive (i.e., non-destructive) sampling to obtain DNA from a vulnerable Singaporean tree frog species.

The phenomenon of global amphibian declines has ushered in a new set of research protocols for performing genetic analyses on endangered and vulnerable species. Many methods for non-destructive sampling techniques have been described. These methods include extracting DNA from buccal swabs (Goldberg et