Assessment of microbranding as an alternative marking technique for long-term identification of New Zealand lizards

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Abstract: 'Microbranding', a system for individually identifying reptiles and amphibians based on a numbered code of spot brands applied to the body and limbs, was tested on New Zealand skinks and geckos. Common geckos (*Woodworthia maculata*) and copper skinks (*Oligosoma aeneum*) were used as test animals. Brands applied in autumn took 3 months or more to heal. There was no evidence of brand-related mortality or increased parasite loads in branded animals. However, after healing the brands faded very rapidly in the skinks to become totally unreadable in all surviving branded skinks after 2.5 years and not accurately readable in most geckos after 3 years. We therefore consider the technique unsuitable as a standard marking procedure for New Zealand lizards.

Keywords: marking mortality; toe clipping

Introduction

Permanent marking of individual reptiles enables the collection of data on longevity, reproductive output, movements, and population size and viability. This information is important in determining the status of a species, and in assisting with predictions on the ability of a species to recover in response to management actions. New Zealand currently lacks a nationally accepted method for permanently marking reptiles. Photographic identification using permanent natural markings can work well in intensively studied populations of those species with naturally variable markings, but is likely to be very difficult after intensive study ceases and many new animals enter the population. Marking techniques for reptiles, amphibians and marine mammals were reviewed in detail by Mellor et al. (2004) and Beausoleil et al. (2004), who found the only permanent marking techniques available for lizards to be toe-clipping, implantation of passive integrated transponders (PITs), and branding. Use of implanted PITs is limited to the largest lizards. The recent release of smaller PITs (http://www.microchips.com.au/products/trovan-nanotransponder) will reduce the minimum size of animals in which the method can be used, but size limits have yet to be established and size is still likely to exclude even adults of the smaller species, and juveniles of most species. The traditional technique of toe-clipping is increasingly being regarded as unacceptable by some iwi (indigenous Māori people's tribal organisations), and this means the New Zealand Department of Conservation (DOC) is unable to issue permits for toe-clipping of protected native lizards in certain areas. As a result, there are locations in New Zealand where rigorous long-term population analyses of threatened reptile species are unable to be done.

Beausoleil et al. (2004) discussed a procedure called 'micro-branding' as a potential marking technique for skinks and geckos. Microbranding was developed in Australia for mark–recapture study of medium-to-large (snout–vent length (SVL) up to 100 mm and weight to 28 g) knob-tailed geckos, *Nephrurus deleani* (Ehmann 2000). Ehmann followed microbranded animals for 4 years, detecting no loss of brand marks, and also branded hatchlings weighing about 1.5 g, following them until they reached maturity. He also tested the technique on other species of Australian geckos, skinks, snakes and frogs, and followed microbranded small skinks in a garden study for up to 3 years, observing no ill effects from the brands (Ehmann 2000).

The technique involves applying a pre-selected heat brand in the form of 'dots' to the upper surface and/or legs of the reptile. The position of the dots is translated into a unique number that identifies a particular animal. We tested this technique on skinks and geckos in captivity to assess its safety and usefulness for marking wild populations. The tests focused on the longevity of the marks, and also assessed the health and survival of the animals following the procedure.

Methods

One of us (SC) developed an inexpensive, easily used microbranding tool based on the one used by Ehmann (2000). The tool consisted of a Mag™ light torch with a nichrome wire replacing the bulb. The wire was wound into a two- or three-loop tip. The two tails of the wire fitted into the two-pin plugs. The tool was powered by 2 AA batteries, which heated the wire. The three-loop wires were used to microbrand the adult geckos and the two-loop wires for the skinks and subadult geckos; the tips on the loops had total branding areas of 2 × 1 mm and 1 × 1 mm, respectively.

Before trials on live animals, tests were carried out by KN and NL on preserved specimens to (a) identify the location of major blood vessels in the legs and (b) practice the application of the branding tool (pressure and length of application). Dissections of preserved copper skinks indicated that to avoid possible damage to major blood vessels, microbranding sites should avoid the leading edge of the front legs and the middle and trailing edge of the hind legs. Dissections of common geckos revealed that microbranding sites should not be placed on the leading edge of their front legs and the trailing edge or close to the knee area of their hind legs.

Common geckos (*Woodworthia maculata*) were collected from Mana Island near Wellington, and copper skinks (*Oligosoma aeneum*) from Great Barrier Island (Aotea Island) (hereafter Great Barrier Island). Both species have had recent name changes (Chapple et al. 2009; Nielsen et al. 2011), the skink being formerly known as *Cyclodina aenea* and the gecko as *Hoplodactylus maculatus*. These species were selected because they are not threatened and were locally abundant, and because the copper skink is the smallest New Zealand lizard species known at the time the experiment started, and was therefore likely to be the most difficult to handle, brand and read brands on, making it a robust test-case for the technique. (The slight skink *Oligosoma levidensum*, split from *O. aeneum* by Chapple et al. (2008), and the recently distinguished pygmy gecko *Woodworthia* sp. (Nielsen et al. 2011) are smaller.)

Ten geckos of each sex were housed in pairs indoors under high-UV reptile lights at Hamilton Zoo in purpose-built wooden and mesh enclosures containing peat moss, leaf litter, small branches, and wooden retreats. Copper skinks (SVL > 50 mm) (n = 10) were housed indoors in pairs in glass aquaria containing peat moss, sphagnum moss, leaf litter and wooden and concrete cover objects, and covered with mesh lids. Reptile lights were placed above the enclosures. All skinks and geckos were split into sex/age groups and then randomly assigned to a treatment or non-treatment group. One treatment and one non-treatment individual were paired in each separate enclosure.

Microbrands were applied to the trial skinks and geckos at Hamilton Zoo in April 2005 by KN, using the numerical code of Ehmann (2000) (Fig. 1). Identification numbers for each pair of lizards were randomly generated in Microsoft Excel. Only numbers between 1001 and 2999 were used, so that each lizard had one brand on one front leg, and most lizards had one or two brands on each hind leg and one or two brands on the body. In reality, most lizards marked in the wild would be given a number less than 1000 (most current studies have sample sizes much smaller than this); however, we wanted to test the 'worst case scenario' of lizards having up to seven microbrands spread over up to three limbs and the body.

Within each lizard pair, the treatment animal was given the brand, and the non-treatment animal was given a 'mock' brand; that is, the animal was handled and the unheated tool applied to the brand sites. For branded animals, the tool was powered for 60 s prior to branding each lizard. Lizards were held in one hand while brands were applied to the body, and the legs were stabilised by a second person gently holding the foot against the holder's hand while the limbs were branded. The tip of the tool was lightly applied to each pre-selected brand site for less than a second. Branding was carried out under the observation of the Hamilton Zoo veterinarian and, in the case of the geckos, of three members of the DOC Animal Ethics Committee.

Brand assessments were carried out on Day 1, 1 week, and 1, 3, 6, 12, 19, 24, 31 and 36 months post-branding. On each assessment occasion, the animals were weighed and measured. Faecal samples were collected from as many animals as possible and screened for internal parasites by BG. After the 31-month check, the surviving skinks (n = 3) were returned to Great Barrier Island, as their brands had become undetectable

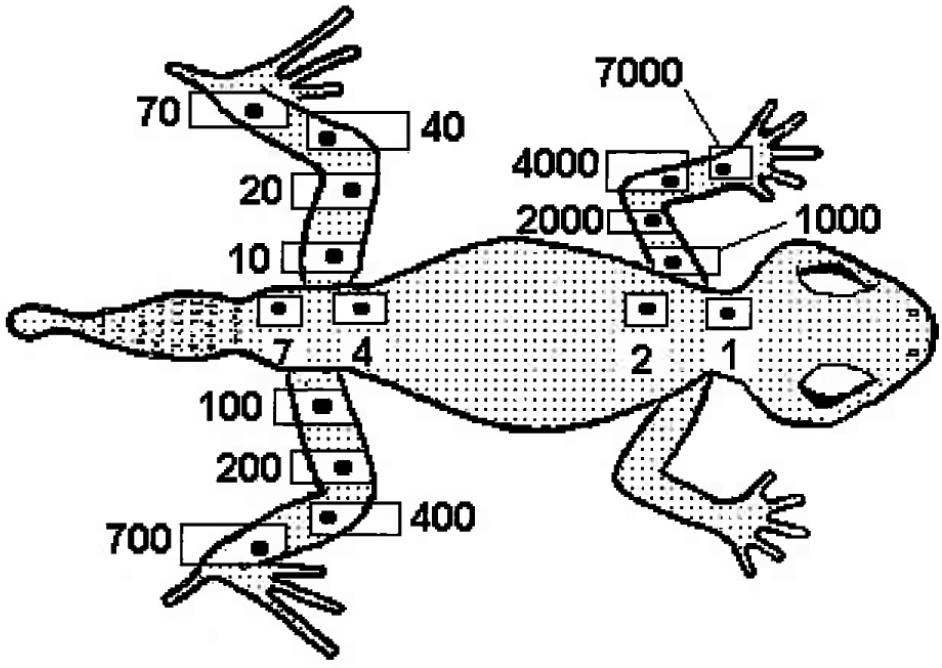

Figure 1. Marking code translator. The number codes for each branding site are added together to give an identification number between 1 and 9999; e.g. animal number 8768 would have a brand on the left forelimb at position 7000 and another at position 1000, a brand on the right hindlimb at position 700, brands on the left hindlimb at positions 40 and 20, a brand on the pelvic region at position 7, and a brand on the nape of the neck at position 1. Reproduced with permission from Ehmann (2000).

and high mortality of both experimental and control animals meant that there was no point in continuing to monitor them. At the final brand check for the geckos, RH (who had been checking the brands over the previous 18 months), KN (who had last seen the brands 2 years earlier in March 2006), and Lynn Adams from DOC's Wellington Conservancy (who had had no previous direct involvement in monitoring the lizards) all assessed the brands. All brand checks were done in the veterinary clinic at Hamilton Zoo using a jeweller's loupe (head-mounted magnifying glass), except for those done by Lynn Adams, which were carried out in the laboratory at DOC National Office in Wellington, using a jeweller's eye-piece.

Any animals that died and were found before their bodies were too deteriorated were necropsied by BG. On each occasion when the live animals' brands were checked, the experimental and control skinks and geckos were held individually overnight in an empty ice-cream container containing a clean, damp paper towel. They were fed wax moth larvae to encourage them to defecate, and all faecal pellets were collected and sent to BG for parasitological assessment.

Results

Common geckos

Five adult and five subadult common geckos were branded on 20 April 2005. The geckos' responses to the application of the branding tool ranged from no response to a flinch and short struggle. There was some variation in the healing rates and results of the branding between geckos (Table 1). Overall, the brand marks were slow to heal. After 1 month, the brands were generally raised and crusty, with some having overlying white epithelium. At 2 months, there had been little change, although most brands looked ready to slough. At 3 months, all the gecko microbrands were able to be identified with the use of a loupe. Those brands that had sloughed had smooth, shiny, unpigmented skin. However, half of the geckos still had crusty and/or raised brand marks. We detected little difference between experimental and control animals in survival, weight gain, or parasite load.

Some poor or ambiguous placement of brands required the identification numbers to be changed, and would have caused problems in a field study. However, most brands were still readily detectable after 2.5 years (Table 2). Nevertheless, a significant number of brands were difficult to detect from 18 months on. There was a general trend for detectability of brands to gradually decrease over time, and brands were particularly difficult to read on geckos that were about to slough. One gecko had brands that from 3 months post-branding onwards were very difficult to see and would likely have been missed in the field. At 2.5 years post-branding, four of the seven surviving marked animals had one or more undetectable brands, meaning their numbers were misread, although they were identified as marked animals. The number of accurately read brands

Table 1. Observations of microbrand marks on common geckos (*Woodworthia maculata*) up to 3 months after branding.

Gecko ID	Day 1	Week 1	1 month	2 months	3 months
HM1	Skin pale, looks like a pre-slough	Unchanged	Slightly crusty, dark with overlaid white epithelium	Crusty, proud. Looks ready to slough	Shiny, smooth, loss of pigmentation
HM7	Skin pale, looks like a pre-slough	Unchanged	Raised and crusty	Actively sloughing, marks shiny, smooth, loss of pigmentation	Still sloughing. Marks shiny, smooth, loss of pigmentation; animal died 2 August 2005
HM11	Skin pale, looks like a pre-slough	Very pale marks, difficult to see	Raised and crusty	Raised, looks ready to slough	Raised and scaly, looks ready to slough
HM14	Skin pale, looks like a pre-slough	Unchanged	Raised and crusty	Raised, looks ready to slough	Dark and crusty, loss of pigmentation in some areas
HM18	Skin pale, looks like a pre-slough	Marks sloughing	Loss of pigmentation, raised and crusty	Marks raised and proud of surrounding skin, looks ready to slough	Marks smooth, shiny
HM19	Skin pale, looks like a pre-slough	Unchanged	Crusty	Crusty, proud, looks ready to slough	Dark and crusty, not yet sloughed
HM21	Skin pale, looks like a pre-slough	Some marks starting to slough	Slightly raised, dark, with white overlying epithelium	Marks sloughing at edges, proud, discoloured	Slightly crusty with dark discolouration
HM22	Skin pale, looks like a pre-slough	Unchanged	Slightly raised, crusty	Dark, looks ready to slough	Dark in centre, loss of pigmentation on outer edge
HM23	Skin pale, looks like a pre-slough	Unchanged	Slightly raised	Sloughing	Loss of pigmentation
HM25	Skin pale, looks like a pre-slough	Unchanged	Dark, slightly crusty	Sloughing, slightly proud, discoloured	Dark, slightly crusty, with loss of pigmentation on outer edge

continued to fall between 2.5 and 3 years post-branding. The experienced observers (KN and RH) correctly read brands on only two of the seven remaining experimental geckos (the same two individuals for both observers), and the inexperienced observer read only one brand combination correctly (Table 2). Brands became undetectable more quickly in subadult than adult geckos (Table 2).

Skinks

Five adult skinks were branded on 27 April 2005. Response to the branding was similar to that of the geckos – ranging from no response to a short struggle. Brand marks initially appeared as two dark brown 'singes' on the skin of the skinks (Table 3). However, the brands became raised and crusty between 1 week and 1 month post-branding. Healing was slow and the skinks' brand marks were not as consistently easy to read 3 months on as they were in the geckos. Some brands resulted in only subtle changes to the skinks' skin pigmentation, whereas others resulted in large areas of unpigmented skin (Table 3).

We detected no significant differences between experimental and control animals in survival, weight gain, or parasite load. Only three skinks survived to the 31-month check: two non-treatment animals and one branded animal on which the brands had become undetectable. Mortality appeared to be lower for skinks held alone in enclosures than for those kept in pairs, so may have been related to social stress.

For the first 6 months post-branding, most skink microbrands were able to be identified with the use of a loupe, but by 3 months, only three of the five branded skinks had brands that were all readily detectable; after that, brand detectability dropped very rapidly (Table 4). The brands on the three treatment skinks that survived longest had all completely disappeared before the skinks died or were released.

Discussion

Microbranding was tested by DOC as a potential alternative marking method to toe-clipping for long-term identification of lizards. The tool developed by SC for this purpose is relatively cheap (about $50) and easy to use. The tool's limitations include not being able to know the temperature of the tip at any time, not knowing if the tool is switched on or off, and possibly inconsistent heat application.

The technique itself is quite quick and easy to carry out. The branded lizards mostly had minor or no visible responses to the hot brand when it was applied. The numbering system is easy to follow, although there could be problems with interpretation of brands on very small skinks (such as copper skinks), or brands that are applied in a position between the prescribed sites.

Branding as a marking tool was not successful for copper skinks, with some of the brand marks quickly becoming difficult to see – despite their being quite blistered and crusty while healing. Two out of five skinks could not be confidently identified at 3 months post-branding. Over time, the number of

Table 2. Accuracy of reading brand combinations of common geckos (*Woodworthia maculata*) from 6 months to 3 years post-branding. Brands that were detected only after the observer knew they should be there are excluded. + = correct number combination. For readings where the individual total number was read incorrectly because not all brands were detected or some had the wrong number code assigned, results are presented as: (number of brands detected/number with position code read correctly). Identities of observers: KN = Keri Neilson, RH = Rod Hitchmough, LA = Lynn Adams.

Gecko ID	Age and sex	Number combination	Number of microbrands applied	KN 6 Oct. 2005	KN 29 Mar. 2006	RH 8 Nov. 2006	RH 11 Apr. 2007	RH 14 Nov. 2007	KN 30 Apr. 2008	RH 30 Apr. 2008	LA 11 May 2008
HM1	Adult male	2291	5	+	+	+	+	(4/4)	+	+	+
HM7	Subadult	2194	5	Dead							
HM11	Adult male	2186	6	+	+	+ (1 extra)*	+	+ (1 extra)*	+	+	(3/2)
HM14	Subadult male	1881	6	+	+	(4/4)	(5/5)	(5/5)	(6/5)	(6/5)	(0/0)
HM18	Subadult female	2688	7	+	+	(4/4)	(6/6)	(6/6)	(6/6)	(6/6)	(6/6
HM19	Adult male	2492	5	+	Dead						
HM21	Adult female	2961	6	+	+	(4/4)	(6/4)	(6/4)	(6/4)	(6/5)	(6/4)
HM22	Adult female	1482	5	+	+	+	+	Escaped			
HM23	Subadult female	1482	6	+	+	(3/3)	+	(5/5) (1 extra)*	(5/5)	(5/4)	(3/3)
HM25	Subadult female	1706	4	+	+	+	+	+	(2/2)	(2/2)	(2/2)
	Detectable†/ Total brands		55	50/50	45/45	35/45	43/45	35/40	36/40	36/40	25/40
	Detectable†/ Total brands – adults		27	27/27	22/22	20/22	22/22	16/17	17/17	17/17	14/17
	Detectable†/ Total brands – subadults		28	23/23	23/23	15/23	21/23	20/23	19/23	19/23	11/23

* Natural scar misread as additional brand.
†Brands that were detected but read incorrectly are included, as many of these errors were due to poor positioning of the original brands.

Table 3. Observations of microbrand marks on copper skinks (*Oligosoma aeneum*) up to 3 months after branding.

Skink ID	Day 1	Week 1	1 month	2 months	3 months
CA5	Singe at brand point	Blistered, some cracked with seepage of serum	Some discoloured, raised and crusty; others difficult to see	Some loss of pigmentation; some raised, others difficult to see	Some crusty, dark colouration; others difficult to see, particularly because animal is sloughing
CA9	Singe at brand point	Discoloured, crusty, some seepage	Slightly pink; flush or concave to skin surface	Leg marks shiny and unpigmented; body marks crusty	Loss of pigmentation
CA12	Singe at brand point, point 700 quite deep	Discoloured, crusty, raised, some cracked	Pink, loss of pigmentation	Slightly raised	Crusty, dark, loss of scales
CA22	Singe at brand point, wounds clean	Dark, crusty, some red underneath	Some loss of pigmentation; others a dark lesion concave to skin	Some shiny and loss of pigmentation; others raised and dark	Pinkish, loss of pigmentation; difficult to see when animal sloughing
CA23	Wounds visible under magnification, difficult with maked eye	Crusty and discoloured, not weeping; limbs moving fine	Pink, loss of pigmentation, flush with skin; scabs sloughed off, clean and tidy	Shiny and unpigmented	Diffuse discoloration, loss of scales

Table 4. Accuracy of reading brand combinations of copper skinks (*Oligosoma aeneum*) from 6 months to 2.5 years post-branding. Brands that were detected only after the observer knew they should be there are excluded. + = correct number combination. For readings where the individual total number was read incorrectly because not all brands were detected or some had the wrong number code assigned, results are presented as: (number of brands detected/number with position code read correctly). Observer initials as in Table 2.

Skink ID	Sex	Number combination	Microbrands applied (*N*)	KN 25 May 2005	KN 13 June 2005	KN 1 Aug. 2005	KN 6 Oct. 2005	KN 29 Mar. 2006	RH 8 Nov. 2006	RH 11 Apr. 2007	RH 14 Nov. 2007
CA5	Male	2861	6	(3/3)	(4/4)	(3/3)	+	(2/2)	(2/2)	(0/0)	Disappeared
CA9	Female	2968	7	+	+	+	+	(5/5)	(1/1)	(1/1)	(0/0)
CA12	Female	1760	4	+	+	+	+	(3/3)	(2/2)	(0/0)	Disappeared
CA22	Male	1488	6	+	+	+	+	Disappeared			
CA23	Male	1850	5	+	+	+	+	(2/2)	Died		

skinks was reduced by mortality in both the treatment and non-treatment groups. The general trend in the surviving branded skinks was for the brands to fade until they were undetectable after 18 months to 2 years. This outcome contrasts with that of Ehmann (2000), who found that, in a back-yard Sydney study, small skinks were unaffected by the brands and survived for at least 3 years (by implication, with the brands intact and identifiable). Ehmann (pers. comm.) relied on changes in scale structure rather than colour changes at the microbrand sites to detect marked individuals. However, we found that in the skinks there were no obvious differences in scale size and shape between the brand sites and surrounding skin once the healing and scale regeneration process was complete.

We have concerns about the use of this technique on small skinks. Even with a small tip on the branding tool, the wound and scar associated with just one brand will take up a large proportion of a small skink's limb. We would not be comfortable putting two brands on the same part of the leg in these skinks (e.g. on the upper leg near the body, and upper leg near the knee). This can be required with the numbering system used, but would result in the entire upper or lower leg being scarred. For these reasons we did not carry out planned microbranding of subadult copper skinks.

For geckos, the technique appears to be somewhat more effective. Three months post-branding, all the gecko microbrands were able to be identified with the use of a loupe, and many brands were still readily detectable after 3 years. However, a significant minority of marks were difficult to detect from 18 months on, meaning that a few marked animals would be missed completely in field studies, and many would be likely to have their numbers misread. Even the more obvious brands can only be identified by examining them under magnification, and are readily missed by people with no experience of looking for them against the geckos' naturally mottled markings. RH had some difficulty reading the brands in his first session after taking over the checking from KN, but then found them easier to detect and was able to read the number combinations more accurately on subsequent checks. Similarly, the inexperienced observer was less accurate than KN and RH at reading brands in the final check (see Table 2). For these reasons the branding technique, while showing some promise for geckos (and, possibly, larger skinks), is not

considered accurate or robust enough to be used routinely in field studies, particularly when people other than the person who did the branding may be checking the brands. Also, it is unlikely to be useful for long-term follow-up of New Zealand geckos, which can live for at least three or four decades (Anastasiadis & Whitaker 1987; Thompson et al. 1992; Lettink & Whitaker 2006). The brands are too subtle to be noticed if they are not specifically being looked for. However, brands would be more obvious on uniformly coloured species; our trial species (*Woodworthia maculata*) has a heavily blotched and speckled colour pattern.

We also have ethical concerns about the technique. Its short-term impact on the animals appears to be more drastic than that of toe-clipping. Up to eight brands per animal may be needed. Each is 1–2 mm wide. On small lizards these wounds are far larger than those left by removing toes, and more brand sites are required per animal than are needed in most toe-clipping studies. The brands are also proportionally much larger than those traditionally used on large domestic mammals – they are only 'micro' because of the 'micro' size of the animals being branded.

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