

# Assessing Hot-Iron and Cryo-Branding for Permanently Marking Southern Elephant Seals

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The process of marking otherwise indistinguishable animals as individuals (and following them through subsequent phases of their lives) has been central to numerous advances in animal ecology, management, and conservation (Caughley and Gunn 1996). Indeed, because accurate survival estimates are important for assessing the conservation status of animals, the quality, durability, and readability of marks applied are essential characteristics that must be taken into consideration when choosing a marking technique. When identifying marks are lost, survival estimates are biased (Eberhardt et al. 1979, Seber and Felton 1981, Frazer 1983, Lebreton et al. 1992); therefore, permanent and legible marks, such as brands, often represent more valuable approaches. There are several other methods of marking animals (Erickson et al. 1993), but some of these methods are either temporary (e.g., tags) or the identifiers may become difficult to read over time. For example, passive integrating transponder tags require all unmarked animals to be scanned with a tag-reading head placed close (usually within 20 cm) to the animal's body so the tag signal has a good chance of being received, and flipper tags require the observer be close to the animal (Clarke and Kerry 1998, Galimberti et al. 2000). Such problems in large vertebrates are largely overcome by branding because the marks are external and visible from a distance (Harwood et al. 1976, Harkonen et al. 1999, Pomeroy et al. 1999, Harkonen and Harding 2001, Raum-Suryan et al. 2002).

The southern elephant seal (*Mirounga leonina*) is one species of large vertebrate marine predator that has provided important ecological, behavioral, and conservation information for Antarctic ecosystems through the marking of individuals (Hindell et al. 2003, McMahon et al. 2003, Bradshaw et al. 2004, McMahon and Burton 2005). Additionally, many southern elephant seal populations have declined in recent times (McMahon et al. 2005a,b), so long-term assessments of their status continue to be required. The Australian Antarctic Division hot-branded southern elephant seals at Heard and Macquarie islands in the 1950s and

1960s (Chittleborough and Ealey 1951, Ingham 1967) and again in the 1990s (van den Hoff et al. 2004) for demographic studies. However, none of those studies evaluated the long-term effects (i.e., survival probability) hot-iron branding may have on the elephant seals being studied. Therefore, we assessed the consequences of branding on survival by comparing estimates of survival probability for branded versus tagged-only seals. We also assessed the effectiveness of cryo-branding because some research suggests that this type of branding may be less invasive than hot-iron branding (Schwartzkopf-Genswein et al. 1997b).

## Study Area

We conducted our study at Macquarie Island (54°30'S, 158°50'E) 1,500 km southeast of Tasmania and 1,300 km north of the Antarctic continent. Macquarie Island is a 34-km-long and 5-km-wide (at its widest point) portion of exposed oceanic crust resulting from uplift within the Macquarie Ridge Complex south of New Zealand. Macquarie Island is the only significant breeding ground for the southern elephant seal in the Pacific sector of the Southern Ocean.

## Methods

Approximately 20,000 elephant seal pups are born each year on the beaches at Macquarie Island between September and November (Laws 1994). Each year for a period of 7 years (1993–1999) approximately 2,000 of these pups were hot-iron branded at weaning (van den Hoff et al. 2004). Of the 2,000 seals branded each year, 1,000 were double-tagged with uniquely numbered plastic tags in their hind flippers within 24 hours of birth (McMahon et al. 1997, 1999). The flipper tags were inserted into the inter-digital webbing between the first and second digits, approximately 25 mm from the trailing edge of both rear flippers. Accordingly, approximately 7,000 pups (1,000 per annum) had double markings (brands and tags), enabling confirmation of our brand and tag recaptures. In 1996 and 1998 an additional sample of seals from each year cohort was tagged as part of a

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study investigating the effects of human disturbance on elephant seals (Engelhard et al. 2001).

### **Branding**

We hot-iron branded weaned elephant seal pups with a 3-digit number and a letter prefix on each side of their caudodorsal flanks (Fig. 1). The letter prefix denoted membership to a particular cohort, and the number uniquely identified the individual. We used 50-mm cast-iron cattle brands similar to those used in previous studies at Macquarie and Heard Islands (Chittleborough and Ealey 1951, Carrick and Ingham 1962, Ingham 1967). We ground all sharp ends and corners on the cast brands to curved profiles and mounted the brands in carriers attached to a 780-mm-long mild-steel rod handle. We heated brands over a gas-fired (liquid petroleum gas) brazier that was protected from the wind by a metal housing that was mounted on the back of a tractor. We positioned the tractor as close to the seals as possible to reduce heat loss from the brands as they were carried from the brazier. A team of 4 people restrained and branded the seals. Two people restrained the seal, one straddling the head and restraining the fore flippers and the other restraining the hindquarters. The third person heated, changed brand characters, and branded, while the fourth person acted as a relief brander, kept records, and applied pressure to the flank opposite to that being branded. We heated brands until they were glowing bright cherry-red in color (approx. 900°C; Böhler Bros. annealing color chart, Kapfenburg, Austria). We monitored all temperature changes by the change in color of the cast iron as given in the annealing color chart. Before branding, we brushed the seal's skin to remove dirt and sand.

We held hot-brands on 12,000 seals with even pressure on the skin for 3 seconds for the first brand-mark and 4 seconds for the second brand-mark to compensate for an approximately 100°C reduction in iron temperature (change from bright cherry red to cherry red–800°C) as the branding irons were moved to the opposite side of the seal. In 1994 only, we marked 2,000 seals with cherry-red brands for 2 seconds on both flanks. After branding, we released the seals and left the burns to heal naturally (see van den Hoff et al. 2004).

In 1991 we cryo-branded 50 weaned pups using 55-mm-wide and 65-mm-high cast-brass brands cooled in either dry ice or liquid nitrogen, and we applied these brands to an area of skin that we shaved of hair. We individually branded 20 seals with 2 numerals cooled in dry ice, and we individually branded 20 seals with 2 numerals cooled in liquid nitrogen. We branded these 40 seals for 10 seconds. We individually branded another 10 seals for 30 seconds. We chilled 5 of these seal brands with dry ice, and we cooled the other 5 in liquid nitrogen. We double-tagged all 50 seals with 2 uniquely numbered flipper tags in the inter-digital webbing of the hind flippers as described earlier.

### **Recapturing Marked Individuals**

We made near-daily surveys of the isthmus to record the presence of branded and tagged seals. We made further searches of the northern third of the island every 10 days,

and we searched the remaining coastline monthly (McMahon et al. 1999). We followed this search regime because there were strong indications that elephant seals at Macquarie Island were philopatric to the northern third of the island. Consequently, we expected the largest number of marked animals to return to the northern end of the island. We standardized searches by following the same route on each occasion, starting the search at the same time on each search occasion and inspecting every seal to determine whether it was marked by brand, by tag, by brand and tag, or not at all. Whenever possible, we read tags and brands without disturbing the seal, but when necessary we spread overlapping hind flippers to facilitate the detection and reading of tags.

### **Analytical Methods**

To quantify the effect hot-branding has on seal survival we compared the tagged-only seal survival estimates to the branded and tagged seals. We calculated first-year survival estimates for marked cohorts using the mark-recapture program MARK (White and Burnham 1999). We compared the survival estimates of 1,000 tagged and branded seals (in 1996 and 1998) with the concurrently tagged-only seals in 1996 ( $n = 108$ ) and 1998 ( $n = 171$ ). We used the likelihood-ratio tests (LRT) in MARK (White and Burnham 1999) to test for survival and recapture differences between branded and tagged seals and also between wounded and unwounded seals. We compared survival estimates for wounded and unwounded seals because, although we recognize that branding itself is a wound, additional wounds may result from the branding process (Chittleborough and Ealey 1951, Csordas 1964, van den Hoff et al. 2004). We defined wounding as splits or cuts in the skin as a result of branding (see van den Hoff et al. 2004 for an image of a typical brand wound). We quantified the effect of such wounds on the seals and compared survival estimates of seals with wounds ( $n = 188$ ) with estimates for seals without wounds ( $n = 1,425$ ). We used LRT to compare 2 nested models (i.e., when the reduced model was a constrained case of the general model). The LRT tests the null hypothesis that the survival rates were constant between groups versus the alternative hypothesis that the survival rates varied between groups (e.g., branded vs. tagged seals and wounded vs. unwounded seals). We used cumulative age-specific tag-retention rates estimated from double-tagged individuals (Pistorius et al. 2000) to adjust all survival estimates to compensate for tag loss. We assumed tagging had no effect on first-year survival, as was the case for weaned Hawaiian monk seal pups (Baker and Johnaas 2002). We standardized the recapture effort between the branded and tagged-only seals by using only the first 18 months of recaptures of each cohort.

All our study procedures were first approved by the Antarctic Animal Care and Ionising Radiation Usage Ethics Committee (Department of the Environment, Commonwealth of Australia) and the Tasmanian Parks and Wildlife Service.



**Figure 1.** A 5-year-old male elephant seal branded B718 in 1993 lays on the periphery of a group of unmarked southern elephant seals moulting on a beach at Macquarie Island (54°30'S, 158°50'E). Note the large (100-mm-high) 4-character caudo-dorsal flank brand that clearly identifies this seal. The letter prefix denotes membership to a specific cohort, while the numerals uniquely identify the individual seal. This seal was hot-iron branded for 3–4 seconds at weaning, producing a clear and legible marking that can be seen from some distance.

## Results

As the seals grew, so too did the brands. At 5 years of age the initial 50-mm-high brand was approximately 100 mm high (Fig. 1), making the seals easily identifiable from a distance. Applying the brands for only 2 seconds in 1994 produced a clear brand at the time of branding; however, after the first molt, the resultant brand was far less discernible from the background pelage than brands applied for 3–4 seconds in other years between 1993 and 1999. Hot-iron branding was 98% effective; only 103 (2%) of 4,862 branded and tagged seals recaptured between 1993 and 1999 could not be identified from their brands alone.

We determined that the first-year survival estimates of branded elephant seals in 1996 and 1998 (69.9% and 67.4%, respectively) were significantly higher than those for tagged-only seals ( $\chi^2_4 = 0.006$ ,  $P = 0.0074$ , 62.1% and 60.3%, respectively). To further assess the effects of hot-iron branding, we compared the first-year survival rates of seals that were wounded at branding and those that were not. The occurrence of brand-associated wounds did not affect seal survival estimates (Wounded: 67.34%  $\pm$  0.02, Unwounded: 67.43%  $\pm$  0.01,  $\chi^2_3 = 0.006$ ,  $P = 0.90$ ). We compared the recapture rates for branded to those for tagged-only individuals because higher recapture rates may lead to higher estimates of survival and because brands are more visible than tags. The recapture probabilities for the 2 groups in the 1996 and 1998 cohorts were similar (Table 1) and we were unable to detect any significant difference in the recapture rates ( $\chi^2_6 = 12.623$ ,  $P = 0.05$ ). Similarly, the recapture probabilities for the 2 wounded groups were not different ( $\chi^2_2 = 0.004$ ,  $P = 0.99$ ).

Of the 50 seals cryo-branded in 1991, we recaptured 26 in following years but we could only identify them by their

flipper tags. No brand marks were visible on any of the 26 cryo-branded seals. Of these 26 seals, 22 had been cryo-branded for 10 seconds, and 4 had been cryo-branded for 30 seconds. We recaptured 18 of these 26 surviving seals (i.e., seals that we observed) between January 1993 and March 2000. We recaptured 8 in the year following branding.

## Discussion

The survival of southern elephant seal pups marked during their first year of life is not compromised by the effects of hot-iron branding compared to flipper tagging. To our knowledge this currently is the only study to quantify the effect that hot-iron branding may have upon the future survival of a branded individual. Clarke (1971) speculated that hot-branded snakes had higher survival prospects than their clipped counterparts, and Aurioles and Sinsel (1988) observed hot-iron branding did not seem to cause significant mortality in Californian sea lions (*Zalophus californianus*). We cannot compare tagged-only seal survival with unmarked seals; however, we believe, as Baker and Johanos (2002) reported for the endangered Hawaiian monk seal, that tagging has no deleterious effects to the survival of southern elephant seal pups. While we are unable to explain our observations that branded seals had higher survival estimates than tagged-only seals we do know this result was 1) unlikely to be the consequence of mark sightability because we observed similar recapture probabilities for branded and tagged seals, and 2) not due to the loss of tags because we compensated for tag loss in our analysis (Pistorius et al. 2000). Nonetheless the important finding was that the survival probability of branded seals was not inferior to that estimated for tagged-only seals. This is important because the application of durable, long-term

**Table 1.** Percent first-year survival estimates ( $\pm$ SE) for branded and for tagged-only southern elephant seals marked at Macquarie Island. Estimates were corrected for brand and tag loss and for preweaning mortality.

| Cohorts                                | 1996               |      | 1998               |      |
|--|--------------------|------|--------------------|------|
|  | Parameter estimate | SE   | Parameter estimate | SE   |
| Survival estimates for branded seals   | 69.9               | 0.02 | 67.4               | 0.01 |
| Recapture rates for branded seals      | 54.7               | 0.01 | 63.6               | 0.01 |
| Survival estimates for tagged seals    | 62.1               | 0.09 | 60.3               | 0.12 |
| Recapture rates for tagged seals       | 53.2               | 0.1  | 57.7               | 0.1  |
| Survival estimates for wounded seals   | N/A <sup>a</sup>   | N/A  | 67.3               | 0.02 |
| Survival estimates for unwounded seals | N/A                | N/A  | 67.4               | 0.01 |

<sup>a</sup> N/A = not available.

marks to identify individuals is a basic requirement used to provide estimates of demographic parameters such as age structure, survival, longevity, dispersal, and fecundity in wild populations (Caughley 1977).

We found that brand application times affected future brand quality such that short application times on the order of 2 seconds were insufficient to permanently kill the underlying melanocytes and hair follicles, resulting in future hair re-growth over the brand scar area. We immediately recognized that there was little point in subjecting animals to such a procedure when the objective was compromised. Thus, we recommend that brands be applied for 3 and 4 seconds because these brand application times were sufficient to destroy the hair follicles and pigment-producing cells resulting in a clear, tan-colored imprint upon brand application and a permanently bald and depigmented brand-mark (Chittleborough and Ealey 1951, Erickson et al. 1993, Merrick et al. 1996). A quality bald depigmented brand was the result of cell coagulation, necrosis, and the denaturation of collagen in the dermis (Demling and Way 1991, Mann and Heimbach 1995).

Cryo- or freeze-branding has been used at times to mark seals (Troy et al. 1997) and was trialed at Macquarie Island as an alternative to tagging and hot-iron branding. No seals cryo-branded in this study have been identified in subsequent years from their marks. All cryo-brand marks were lost after one year. The failure of the cryo-brands and the success of the hot-iron brands suggests that, at least for southern elephant seals, extreme heat is more effective at destroying melanocytes than extreme cold. This is probably because the demarcation of the necrosis zone (the brand mark) takes place during the first few days after branding (Knabl et al. 1999a,b), and the effects of hot branding are more acute and last longer than those of cryo-branding (Schwartzkopf-Genswein et al. 1997a). Given the failure of the cryo-brand marks in our present study, and the mixed success of the technique experienced by other seal researchers (Merrick et al. 1996, Troy et al. 1997), it appears that cryo-branding is a generally less-permanent technique for marking pinnipeds that has a similar handling and application methodology to hot-iron branding, and that does produce usable identification markings for elephant seals.

Choice of marking method will depend on many different factors, not least of which should be the planned duration of

the project. For long-lived animals such as large vertebrates, the ideal methodology should be one that maximizes the lifespan of the mark to match that of the target species. In this way, long-term life-history parameters such as reproductive success, long-distance dispersal, and longevity can provide insights into the evolutionary context of the target population. Unfortunately, temporary marks rarely provide insight into these processes unless they are repeatedly re-applied over the duration of the individual's lifetime. The hot-iron brands we have applied to southern elephant seals at Macquarie Island produced good-quality readable markings (Fig. 1) on the vast majority of the seals (this study, van den Hoff et al. 2004). The longevity of a quality hot-iron brand is undeniable (Hindell and Little 1988).

Application of permanent marks to animals usually raises ethical and emotional concerns. On the one hand, a permanent mark removes the need to re-apply temporary markings, thus resulting in a reduction in the cumulative disturbance experienced by the marked individual over time (Gentry and Holt 1982, van den Hoff et al. 2004). However, a permanent mark such as a brand requires some initial degree of superficial damage to the animal's tissues but with no further disturbance to the animal during its life to re-apply identifying marks. The increasing levels of scrutiny that management authorities and the general public apply to scientific research has resulted in greater concern for the welfare of wild individuals exposed to invasive experimental procedures. Hot-iron branding of southern elephant seals on Macquarie Island became so politically controversial that the program was suspended indefinitely (Jabour-Green and Bradshaw 2004). Similar political intervention into conservation research programs employing permanent marking methods has been recorded in New Zealand (Ananova 2000) and the United States (Dalton 2005). Therefore, despite the mounting evidence in favor of using permanent marking techniques such as hot-iron branding (in terms of durability, readability, and lack of long-term negative effects that we have demonstrated here) careful consideration of the political environment in which the procedures would take place should be made before managers opt for such methods. It is our suggestion that preliminary studies to ascertain the effectiveness and long-term implications of the method of choice be trialed and analyzed prior to the implementation of a full-scale marking program. After demonstrating the effectiveness (van den

Hoff et al. 2004) and lack of negative impacts (this study) of a particular method, the procedure is much more likely to gain support from objective regulating authorities and the general public.

## Management Implications

Long-lived marks on animals allow researchers unique insights into the lives of wild animals, which include quantifying demographic factors such as survival, reproductive behavior and success, immigration, and emigration. Measuring life-history parameters from long-lived markings provides a mechanism with which to understand how animals respond to short-term fluctuations in climate (e.g.,

El Niño events) and to long-term changes such as those linked to global climate change.

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