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Temporal changes in the quality of hot-iron brands on elephant seal (*Mirounga leonina*) pups

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Abstract. Hot-iron brands were used to mark permanently 14 000 six-week-old southern elephant seal (*Mirounga leonina* L.) pups at Macquarie Island between 1993 and 2000. We assessed temporal changes in the quality of 4932 brands applied in 1998 and 1999 to determine the duration of the brand wound, and the relationships between brand healing, brand readability and the amount of skin and hair damage peripheral to the brand characters. Most (98%) brand wounds were healed within one year. Brand-mark healing, peripheral skin damage and brand readability were significantly ($P < 0.05$) correlated. The proportion of healed and readable brands increased in the population during the first annual moult, and thereafter these proportions remained high (>95%) for the marked population. The mean number of brand characters with peripheral skin damage decreased significantly over the same period. The seal's annual hair and skin moult is the process that contributed most to the healing of brand wounds. We also assessed our branding technique to determine whether any of the features we measured contributed to a poor-quality brand. Excessive pressure used during brand-iron application is the most probable cause of unsightly peripheral skin damage, but this damage is short lived.

Introduction

A population's status is measured by census and through analysis of the demographic parameters, which often requires the identification of individuals. Marked individuals are used to assess a population's age distribution, survival prospects, longevity, fertility and other demographic parameters (Caughley 1977). There are many procedures used for marking animals depending on the study species. Whatever the marking method, the result should be a clear identifier that does not adversely affect the behaviour, physiology, ecology or survival of individuals, with due consideration to the nature and duration of restraint, the amount of tissue damage, the amount of momentary or prolonged pain and the risk of infection (Anon. 1998). Evaluation of the effects on marked individuals is desirable, but the provision of appropriate controls is often difficult. For this reason there are few systematic studies that assess the potentially adverse effects of marking procedures and many of the recommendations for marking methods are based on unpublished observations (Anon. 1998).

One of the first procedures used to mark permanently individuals in populations of seals was hot-iron branding (Scheffer 1950), but the method fell from favour with the introduction of tagging (Erickson *et al*. 1993). However, Gentry and Holt (1982) suggested that injury (slight) to an animal once in its life by branding may be preferable to the stress of repeated captures to refresh temporary markings. Tagging is a semi-permanent marking method and has associated problems – the most often reported being the loss of tags over time (Eberhardt *et al*. 1979; Krebs 1989; Bradshaw *et al*. 2000; McMahon *et al*. 2000), but the fading of tag colour, tag breakage and symbol loss can also occur (Broderick and Godley 1999). Recent passive integrated transponder technology is, at best, useful only as a backup to tagging elephant seals (Galimberti *et al*. 2000). Compared to tagging, hot-iron branding remains the least favoured marking technique but branding appears to be the method most unaffected by the aforementioned problems associated with tagging (Erickson *et al*. 1993; Merrick *et al*. 1996; Galimberti *et al*. 2000).

When first branding weaned elephant seal pups we noticed an undetermined proportion of pups were poorly branded compared to others and, in successive years of observing the same marked seals the proportion of poorly branded seals decreased. We suspected that brand quality had improved over time and poorly branded seals were not lost from the population due to a greater risk of dying from any brand-related wounds. Due to this uncertainty, we established a detailed longitudinal study (i.e. a study following brand characteristics of individual seals over time) to assess

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the variables we suspected might contribute to the initial brand quality, and to determine whether changes in these factors might be responsible, to some extent, for future brand quality. We also examined in detail the brand quality over time on individual seals by assessing temporal changes in brand healing and brand readability, and measuring changes in the amount of tissue damage peripheral to each brand character.

To date there have been no detailed published studies assessing the progression of hot-iron brand healing and readability on individuals of a free-ranging species, nor have any studies evaluated factors at the time of branding that may affect future brand quality. In this paper we examined the notion that systematic changes in brand quality do occur over time, especially during the annual terrestrial moult period when a total replacement of hair and skin occurs (Ling 1965).

Materials and Methods

Study animals and procedures

Four thousand recently weaned southern elephant seal pups were captured on the isthmus of Macquarie Island (54°30′S, 158°50′E) in October and November 1998 and 1999. Each pup was physically restrained while we applied 50-mm cast-iron cattle brands, heated to ‘cherry-red’ with a gas brazier, for 3 s to the first side branded and 4 s on the second side branded. The brands we used were fully formed such that the rounded numbers 0, 6, 8 and 9 and letters P and D (for example) formed complete closed shapes. The time difference for branding each side of the seal was introduced to compensate for heat loss during the first branding (Chittleborough and Ealey 1951; Carrick and Ingham 1960). Due to time constraints and the desire to restrain each seal for as little time as possible, the brands were not reheated between sides. Likewise, having two irons with the same brand heating simultaneously, so that a fresh hot brand could be applied to each side, would have extended the capture time and doubled the amount of equipment required.

We used a four-character, alphanumeric brand consisting of a letter prefix followed by a three-digit number that uniquely identified cohort and individual (e.g. E553, Fig. 1*a*). Of the 4000 seals branded in 1998 and 1999, those branded with a P ($n = 1000$) or T ($n = 1000$) prefix had been tagged at birth with two plastic, four-digit tags (McMahon *et al.* 1997), which were not related to the brand, unless by coincidence. The remaining 2000 seals were not tagged at birth but were branded with an E or U cohort prefix to the numeric code.

From previous research and branding we observed that: (1) female pups weighed less than males at weaning; (2) tooth eruption is observed mainly in females at weaning (McMahon *et al.* 1997) (thus, females may be more effective at biting the person restraining the head end during capture); (3) the black gossamer-like hair of new-born pups that is sometimes still present on moulting weaned pups is highly flammable and will catch fire rather than singe; (4) an undetermined proportion of weaned pups have open wounds over various parts of their body after being bitten by intolerant adult females still suckling their own pup, and these bites may confound brand healing; (5) seals with a wet pelage had clearer brands at the time of application; and (6) there were cohorts (years) upon which brands were more readable than on others.

During the 1998 and 1999 breeding season, we recorded aspects of brands applied to 2466 individual seals marked at Macquarie Island ($n = 1799$ in 1998; $n = 667$ in 1999). At the time of branding we recorded the sex ($n = 2453$), presence/absence of black hair ($n = 2216$), presence/absence of skin lesions caused by seal bites in the brand area ($n = 1548$) and whether the hair was wet or dry ($n = 1542$). We recorded whether the seal moved excessively during restraint (possibly causing the irons to slip and smudge the brand) ($n = 1542$) and whether the heated irons burnt through the skin upon application ($n = 1542$). We also recorded the substratum type upon which the seal was branded ($n = 1551$), since this affects the efficiency of physically restraining a weaned seal pup. Substratum type was either: (1) boulder-strewn shore, (2) relatively level sand and cobble beach, or (3) coastal terraces with mixed *Poa* spp. tussock grassland. To investigate the effect of heat loss from the branding irons during branding, we recorded the side (i.e. right or left flank) branded first for 2012 seals. Due to oversight, the brander’s identity was not recorded at the time of branding. From previous capture events of some weaned pups before their branding for this study, we also had data on their mass ($n = 969$), maximum girth ($n = 516$) and dorsal fat depth ($n = 259$) (see Field *et al.* 2002 for methods).

Daily searches of the isthmus study area and monthly searches of the entire island were made over a two-year period to locate and identify marked seals when they returned for the moult or mid-year haul-out (Hindell and Burton 1988). One or two observers then recorded the quality of both the right- and left-flank brands when visible. Moult periods were divided into four stages by assessment of the seals’ visible surface area: (1) ≤33% moulted, (2) 34–66% moulted, (3) >66% moulted, and (4) completed moult. This measure was not exact but it served to partition the moult period for time-related analyses. In-text abbreviations for these periods are coded as: M1/S1 = first moult/0–33% moulted; M1/S2 = first moult/34–66% moulted etc. through to M2/S4 = second moult completed. The mid-year haul-out is a single, non-moulting period for these young seals.

We assessed brand quality using three criteria: (1) brand readability, (2) brand healing, and (3) skin damage peripheral to the intended brand mark. Brands were defined as readable (i.e. all characters of the brand were identifiable: Fig. 1*a*), or unreadable (i.e. some or all characters of the brand were unidentifiable: Fig. 1*b*). Healing was defined as healed or unhealed. Healed brands did not contain any characters that had branding-associated open wounds (Fig. 1*a*). An unhealed brand contained at least one character where the skin was at least partially broken (Fig. 1*b*). Peripheral skin damage was defined as the presence of an area of burnt hair and/or skin surrounding a brand character. This type of damage can occur around any number of brand characters and so we recorded the number of brand characters (i.e. 0–4) that were affected in this way. A ‘0’ indicated that no characters were affected, ‘1’ indicated that only one character was affected, and so on to a maximum of 4 characters (Fig. 1*b*).

The variables sex, hair type, skin wetness, the brand burning through the skin, bites in the brand area, seal movement, brand readability and healing were recorded at branding and classified into one of two possible outcomes (i.e. the data were binomial). Peripheral skin damage was classified into five categories, and substratum into three types. Seal mass, girth and fat depth were continuous variables.

Analysis

To determine the effects of sex, mass, girth, fat depth, hair type, skin wetness, substratum type, bites in the brand area, side branded first and seal movement on brand smudging and a brand burning through the skin upon application, we used a series of one-way analysis of variance (ANOVA) tests using full-term models only (i.e. ignoring any interactions due to the large number of competing models). We also used ANOVA to determine the effect of each variable measured at branding on future brand readability, healing and the amount of peripheral skin damage recorded during the seal’s first moult. Student’s *t*-tests for dependent samples were used to test for differences between the quality of first and second brands within each time step (M1/S1, M1/S2 etc).

To examine time-related changes in brand quality over the first moult (M1), mid-year haul-out and second moult (M2) we applied a series of repeated-measures analysis of variance (RM-ANOVA) using a univariate design with time as the repeated measure. With much of the data binomially distributed, a normal approximation can be assumed because there were a large number (in most cases >100) of observations contributing to computation of the standard errors (Fowler *et al*. 2000). A series of RM-ANOVA was used because the number of brands that were assessed on all possible sighting occasions (M151 to M254) for any brand factor was small ($n = 4$). First, we compared brands that were seen at M1/S1 and again at M1/S4 and during the mid-year haul-out. Second, we compared brands that were seen in the mid-year haul-out and again at M251 and M254. Using this approach, each model included only brands that were seen subsequently.

Simple linear regression was used to determine correlations between the three brand factors, and also the amount of variation each of the three brand factors contributed to the measured changes in the other.

Graphs with error bars produced from the RM-ANOVA in STATSOFT STATISTICA software do not provide an acceptable graphical test of the significance of the repeated measure; they show only whether there is a significant difference between the groups if they were treated as ordinary dependent samples (without repeated measures) because the RM-ANOVA result takes covariance into account (Richard Fraccaro, STATSOFT, Melbourne, personal communication). Therefore the graphical representation of the RM-ANOVA results does not have bars to represent error or confidence. $P \leq 0.05$ denotes a statistically significant difference.

Results

Brand resighting

Over the two-year study period searching the coastline of Macquarie Island for marked seals, we made 3560 resights of the 2466 seals branded in 1998 and 1999. Seals were seen during each of the observation periods but no single seal was observed ashore in all 10 of the possible periods. Generally, more seals were seen once than seen on multiple occasions (Table 1).

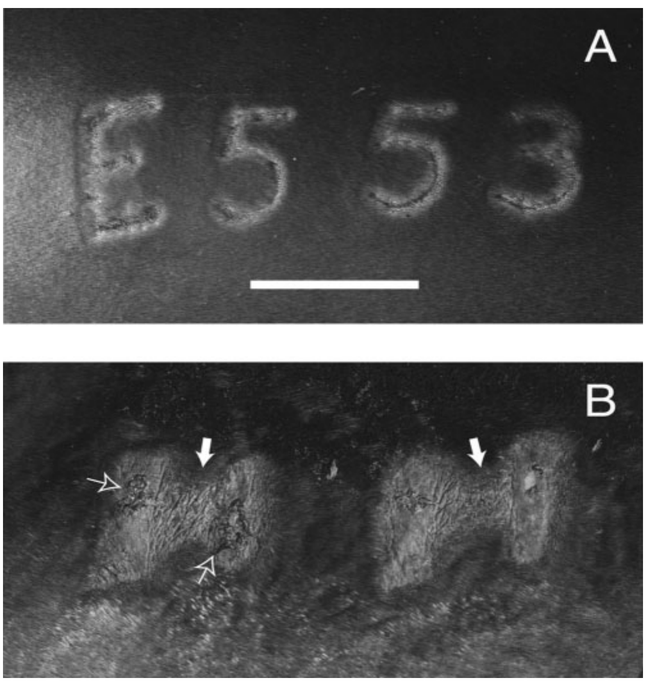

Fig. 1. Examples of differences in brand quality: (*A*) A readable, healed brand with no peripheral skin damage. The white bar represents 50 mm on the seal. (*B*) An 'unreadable' brand (E825) showing some 'unhealed' (open arrows) characters with peripheral skin damage (solid arrows). The brand on seal E825 was determined from the second brand, which was readable.

Upon-application causes of poor-quality brands

Brand smudging

We observed that the incidence of brand smudging was related to seal movement at time of capture, and the branding iron slipping on the seal's flank upon application. Of the 3077 brands assessed on the 1799 seals branded in 1998, 461 brands (15%) were, to some degree, smudged upon application, and smudged brands were significantly related to: (a) the side branded first – significantly more brands that were applied first were smudged ($n = 253$) than those applied second ($n = 208$) (LS means $F_{1,2672} = 11.46$, $P = 0.001$); (b) sex – more male seals had their first-applied brand smudged than females (LS means $F_{1,1526} = 8.93$, $P < 0.01$); (c) mass – the mean mass of weaned pups with smudged second brands (124.4 kg) was heavier than those with a clear second brand (115.8 kg) (LS means $F_{1,455} = 6.67$, $P < 0.01$). (There was no mass difference if the order of branding was ignored. Moreover, sex and mass are related because males were heavier than females.); (d) wetness – a greater proportion of seals with a dry pelage had smudged brands (LS means $F_{1,3077} = 27.831$, $P < 0.001$), and this result was significant for first and second brands applied.

Using a cross-sectional approach and verifying smudged brand characters with either the flipper tag or the second brand that was not smudged we studied time-related changes in the readability and healing of smudged brands. We determined that the readability and healing of smudged brands improved with time. Of the 461 brands smudged during application, 87 were observed again at M1/S1, and, of these,

Table 1. Numbers of southern elephant seals never seen or seen 1–10 times on Macquarie Island in the branding study period between the beginning of the 1998 moult haulout and the end of the 1999 mid-year haulout

A seal could be seen on a minimum of no occasions or a maximum of 10 if it were seen in all the study periods

Possible number of times that a seal might be seen	No. of seals
Not seen	842
1	683
2	413
3	258
4	143
5	74
6	41
7	8
8	3
9	1
10	0

15 (17%) were classified as unreadable. In all, 72 of the original 461 smudged brands were seen at M1/S4, and by then the proportion of smudged brands that remained unreadable in the study population had further decreased to 13%. The proportion of smudged brands that were unreadable continued to decrease with time. In total, 161 of the 461 smudged brands were seen again during the mid-year haul-out; at this time 3% (5 of the 161 brands known to be smudged upon application) were unreadable.

Six (6.7%) of the 87 smudged brands assessed at M1/S1 were unhealed. All of the 72 smudged brands seen at M1/S4 were healed; however, the proportion of unhealed smudged brands that were seen in the mid-year haul-out was still 6% (9 of 158). The proportion of smudged brands with 4 characters showing peripheral skin damage decreased between M1/S1 (n = 97) and the mid-year haul-out (n = 157) from 32% to 7.6%, respectively. At the same time the proportion of characters without peripheral skin damage increased from 26 to 65%.

Brands burning through the skin

Of the 3080 brands assessed in 1998, 102 (3.3%) broke through the skin during application. Skin breakage from branding was significantly related to: (a) wetness – the proportion of brands with skin breakage was significantly greater for brands applied first to wet seals (LS means $F_{1,1535} = 5.42$, $P < 0.05$) than for brands applied first to dry seals; and (b) sex – more brands on male seals (n = 64) had burnt through the skin than had those on females (n = 38) (LS means $F_{1,3060} = 4.922$, $P < 0.05$).

Of the 102 (57 applied first side and 45 applied second) brands that had broken through during application, 10 were observed again at M1/S1; of these, 4 (40%) were classified as unhealed. Thirty-five of the original 102 (34%) brands that broke through during application were seen at the mid-year haul-out, and by then the proportion of brands that remained unhealed had further decreased to 8.5% (3 brands).

The proportion of readable brands in the population of brands that had broken the skin also improved over time as the proportion of unreadable brands decreased from 35% (n = 17) to 5% (n = 35) between the M1/S1 and the mid-year haul-out, respectively. Too few measurements of brands with peripheral skin damage and that had burnt through the skin were made at M1/S1 and M1/S4 for analysis.

Year branded

Significantly more brands that were applied first in 1998 and observed one year later at M1/S1 were readable (LS means $F_{1,823} = 4.183$, $P < 0.05$), healed (LS means $F_{1,816} = 7.952$, $P < 0.01$), and had fewer brand characters with peripheral skin damage (LS means $F_{1,822} = 49.916$, $P < 0.001$) than those that were applied first in 1999. Significantly more brands that were applied second in 1998 and observed at M1/S1 were healed (LS means $F_{1,794} = 22.078$, $P < 0.001$) and had fewer brand characters with peripheral skin damage (LS means $F_{1,797} = 47.991$, $P < 0.001$). Only the readability of the second application of brands did not differ significantly between years ($P > 0.05$).

Pooled first- and second-side assessments of brand readability, healing and peripheral skin damage one year after branding at M1/S1 showed that brands applied in 1998 were in all cases significantly ($P < 0.01$) better than those applied in 1999. At M1/S4, the healing and readability of brands applied in 1998 and 1999 were no longer significantly different ($P > 0.05$); however, the year difference for peripheral skin damage (0.5 ± 0.05 and 1.2 ± 0.05 characters for seals brand in 1998 and 1999, respectively) persisted through the first moult (LS means $F_{1,1036} = 60.82$, $P < 0.001$). No further observations were made to determine whether this difference remained into the next year.

Temporal changes in brand quality for all marked individuals (cross-sectional analysis)

Brand readability

A significantly greater proportion of brands that were applied first (76%) were readable at M1/S1 than were brands that were applied second (71%) (t-test for dependent samples, $P < 0.05$) (Fig. 2). After this time, no further significant changes were detected between the first and second brand applied (Fig. 2). Brand readability at M1/S1 was significantly influenced by the amount of peripheral skin damage (Side 1, LS means $F_{4,818} = 74.17$, $P < 0.001$; Side 2, LS means $F_{4,792} = 65.024$, $P < 0.001$). Generally, brand readability decreased with increasing peripheral skin damage. Brand healing strongly influenced brand readability: unhealed brands were more difficult to read than healed ones (Side 1, LS means $F_{1,816} = 22.754$, $P < 0.001$; Side 2, LS means $F_{1,794} = 26.693$, $P < 0.001$).

Brand wound healing

During the mid-year haul-out was the only period in this study when there was a significant difference ($P < 0.05$) in the healing of first and second brands applied (Fig. 3). This difference, while statistically significant, was detected when more than 95% of the brands observed were already healed.

Healing of brands at M1/S1 was significantly correlated with peripheral skin damage (Side 1, LS means $F_{4,813} = 10.501$, $P < 0.001$; Side 2, LS means $F_{4,788} = 6.677$, $P < 0.001$): fewer brands with skin damage around 4 brand characters were healed than brands with 0 or 1 character with skin damage. In addition, healing of first brands was related to skin breakage upon brand application (LS means $F_{1,315} = 18.40$, $P < 0.001$). Fewer brands were healed at M1/S1 if the skin was penetrated by the brands at branding.

Peripheral skin damage

There were significant differences between the first and second brands in the number of brand characters with peripheral skin damage (Fig. 4): brands applied second tended to have more peripheral skin damage than brands applied first. As well, at M1/S1, peripheral skin damage on the second side branded was significantly (LS means $F_{4,156} = 2.99$, $P < 0.05$) related to a seal's fatness at weaning: fatter weanlings had fewer brand characters with peripheral skin damage. Peripheral skin damage to the first side branded was significantly related to mass (LS means $F_{4,325} = 2.68$, $P < 0.05$): heavier weanlings had fewer brand characters with peripheral skin damage.

Temporal changes in brand quality on individual seals (repeated-measures ANOVA)

Brand readability

Between M1/S1 and M1/S4, the proportion of readable brands in the population increased significantly from 81.6 ± 3.3% to 94.7 ± 1.9% (Time, LS means $F_{2,260} = 19.843$, $P < 0.001$) (Fig. 5). This trend was followed by another, albeit small, increase to 98.5% ± 1.0% of brands observed in the mid-year ($n = 132$). Readability remained high (>95%) from the mid-year haul-out through M2 (Fig. 5), but there was a significant but small decrease in readability from 97.4% in the mid-year to 95.1% at M2/S1 (Time, LS means $F_{2,614} = 3.376$, $P = 0.034$), after which readability improved again to 98.0% by M2/S4 (Fig. 5). Specifically, 132 brands seen as unreadable at M1/S1 were seen again at M1/S4 (4 weeks later) when 18 (13%) remained unreadable. Conversely, only 3 of 378 (0.8%) readable brands were judged unreadable over the same period.

Brand wound healing

In total, 87% of brands seen at M1/S1 were healed (Fig. 5). This proportion significantly improved (Time, LS means $F_{1,506} = 52.934$, $P < 0.001$) to 98% by the end of the first moult and remained <98% for the remainder of the study. Repeated measurements of healing were made for 63 unhealed brands and 444 healed brands. Four unhealed brands remained unhealed during the first moult while four of the 444 healed brands (<1%) became unhealed.

Peripheral skin damage

A significant reduction in the mean number of brand characters with peripheral skin damage occurred between M1/S1 and M1/S4 (1.82 ± 0.14 to 0.5 ± 0.08 characters) (Time, LS means $F_{2,264} = 84.694$, $P < 0.001$) (Fig. 5). Peripheral skin damage remained low (<0.5 characters) at the mid-year haul-out and second-moult period but there was a significant slight change (0.33 characters to 0.47 characters) at M2/S1 (LS means $F_{2,610} = 5.485$, $P < 0.01$) (Fig. 5).

Correlations

Brand healing, brand readability and the number of brand characters with peripheral skin damage were highly corre-

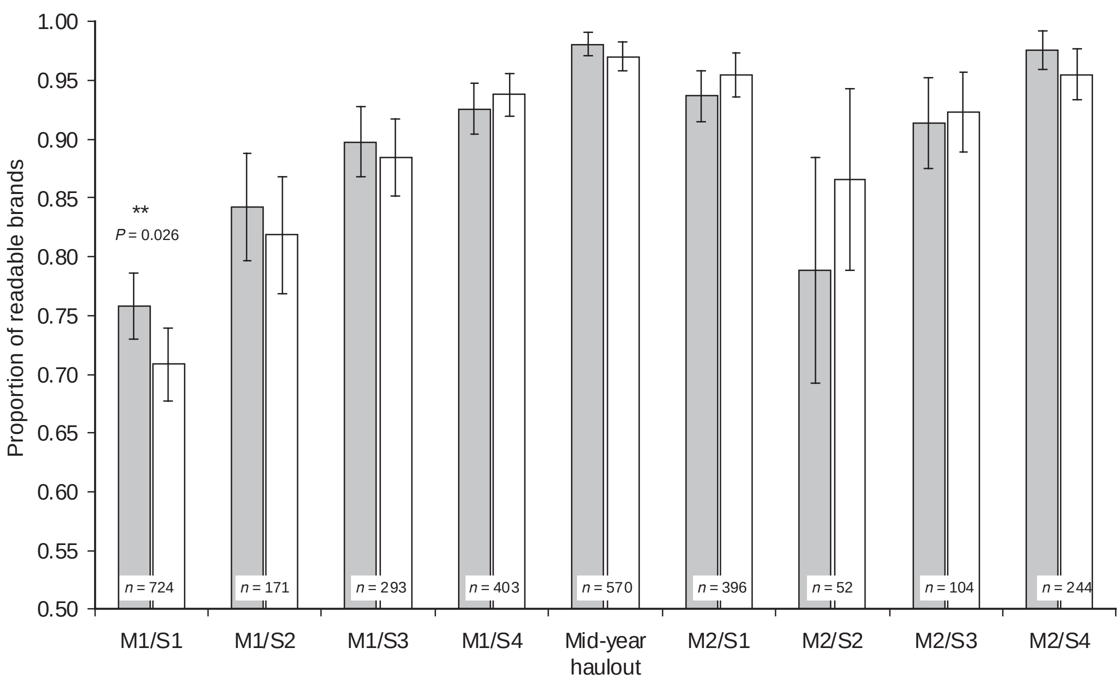

Fig. 2. Change in the proportion of readable brands in the sample population of branded southern elephant seals observed during their first annual moult, mid-year haul-out when the seals are not moulting and the second moult. The dark bar represents the first side branded, the open bar represents the second side branded; error bars above each histogram bar represent 95% confidence limits, and statistically significant differences are shown as ** with probability value. See Materials and Methods for descriptions of codes used.

lated (Fig. 6). When model selection was limited to a single correlation, the r^2 values indicate that 92.5% (t_7 = 176.11, $P < 0.001$) of the variation in the number of characters with peripheral skin damage was associated with brand healing. Much (74.8%) of the variation observed in readability was produced by healing (t_7 = 2.7, $P < 0.05$). As peripheral skin damage decreased, brand readability increased (r^2 = 81.3, t_7 = 42.29, $P < 0.001$).

Discussion

The results of this study conclusively show that systematic improvements in the quality of hot-iron brands placed on a large number of southern elephant seals for an extensive long-term population and ecological study occurred during the seals' first annual moult when the hair and skin are completely shed and replaced (Ling 1965). We observed that after initial brand application most brands on the seal pups were readable (95%) and healed (98%) when the seals were 12 months of age, and these proportions continued to increase or remained high in the following year. We also found that brand healing, brand readability and peripheral skin damage were significantly ($P < 0.05$) correlated (Fig. 6*a*–*c*). The strong correlation coefficients suggest that brand wound healing, which occurs through the moult, is responsible for the associated improvements in brand readability and peripheral skin damage. The changes in recorded brand-quality were so profound that previously unreadable brands became readable and unhealed brand wounds with peripheral skin damage became healed scar tissue over a short period.

The process of hair loss in phocid seals begins while the animals are still at sea (Ling 1965; Ashwell-Erickson *et al.* 1986). When southern elephant seals moult (a process that takes ~16 weeks), the hair and cornified epidermis tissue (skin) are entirely shed and replaced (Carrick *et al.* 1962; Ling 1965). The most significant changes in brand quality occurred in the first 4 weeks of the terrestrial moult period (M1/S1 to M1/S4), and this leads us to conclude that moulting is the process that led to the changes in brand quality that we measured. The main area of a brand itself remained a permanent scar because the hot brand-face was in direct contact with the skin, and thus the underlying hair follicles and pigment-producing cells were killed (Merrick *et al.* 1996). As the moult progressed the already dead cells and follicles were not replaced, but damaged cells and follicles in the brand periphery (peripheral skin damage) that were not killed were regrown. The healing of brands that we observed after one year is consistent with the results of Troy *et al.* (1997), who found that infected (unhealed) brands on New Zealand fur seals (*Arctocephalus forsteri*) persisted for six months but were completely healed within 12 months after branding. Wilkinson *et al.* (2001) found healing and readability of brands on Hooker's sea lion pups (*Phocarctos hookeri*) also improved over 12 weeks.

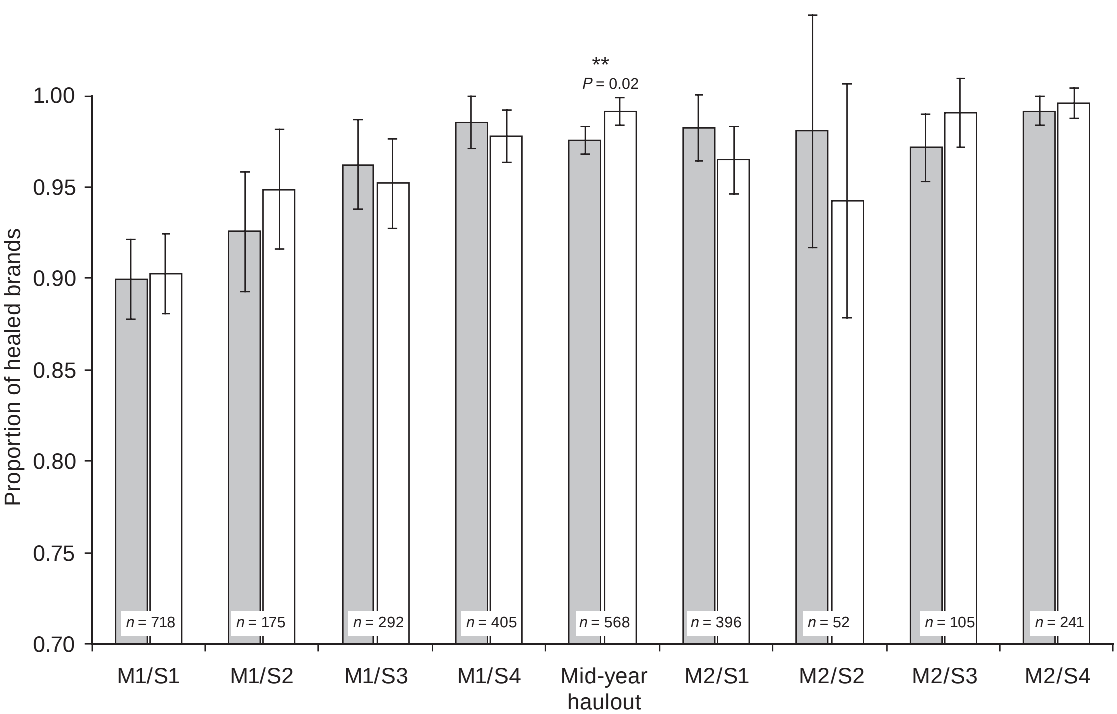

Fig. 3. Change in the proportion of healed brands in the sample population of branded southern elephant seals observed during their first annual moult, mid-year haul-out when the seals are not moulting and the second moult. See Materials and Methods for descriptions of codes used.

As with alternative marking methods, there were several brand markings that were of inferior quality relative to others. Of the factors that we assessed at the time of branding, the year in which the seals were branded most affected brand quality. Year of branding can be used as a measure of differences in methodological application (i.e. different individual branders). The persistence of peripheral skin damage beyond the first moult recorded for the 1999 seals is probably due to between-year differences in brand-application pressure. The skin of an elephant seal pup overlays 40–50 mm of compressible blubber that a wedge-shaped branding iron, like the ones we used, can depress and thus result in a burn peripheral to the intended area if excessive pressure is applied (Fig. 1*b*). Peripheral skin damage was significantly related to the seal's fatness (blubber depth and mass), and was seen most often on the second side branded. Fatter (heavier) pups have a tauter skin surface than less-fat individuals; therefore, the skin of heavier seals may be less likely to rise up around the brand symbols and cause peripheral skin damage. Year differences could then be attributed to annual variation in wean mass; however, none were detected (Australian Antarctic Division, unpublished data). We did not specifically test for differences attributable to the branders themselves so we cannot eliminate individual differences in brand pressure as having an influence on brand quality.

Csordas (1995) identified brand-application pressure as the most probable cause of the peripheral skin damage. When assessed at, or very soon after, branding, peripheral skin damage can make the brand area look far worse than it truly is. However, if the underlying hair follicles and pigment-producing cells remain alive the area of damaged hair surrounding each brand character is replaced after one year. Brand-iron application pressure could be better standardised with the use of a 'constant pressure' spring-based brand holder that would not reduce the efficiency of the process. Alternatively, a different brand profile such as a rounded face rather than a wedge-shape could be trialled to reduce the possibility of a brand breaking through the skin upon application.

The repeated-measures ANOVA we used had the power to identify true temporal trends in brand quality because the models used only brands that were seen at subsequent times (e.g. M1/S1, M1/S4 and the mid-year haul-out). This evidence not only shows that brand quality does improve over time but it is also important evidence to demonstrate that seals with unhealed brands did not disappear from the study population through an increased likelihood of death from their brand wounds; rather, they were more likely to be identified given the healing of previously unreadable marks. Thus, we found no evidence of an increased mortality associated with this marking procedure, a concern raised by McGilvray (2000). Indeed, another study involving a much larger sample of seals (Australian Antarctic Division, unpublished data) showed that the survival probability of seals with poor-quality brands was not lower than that for seals with

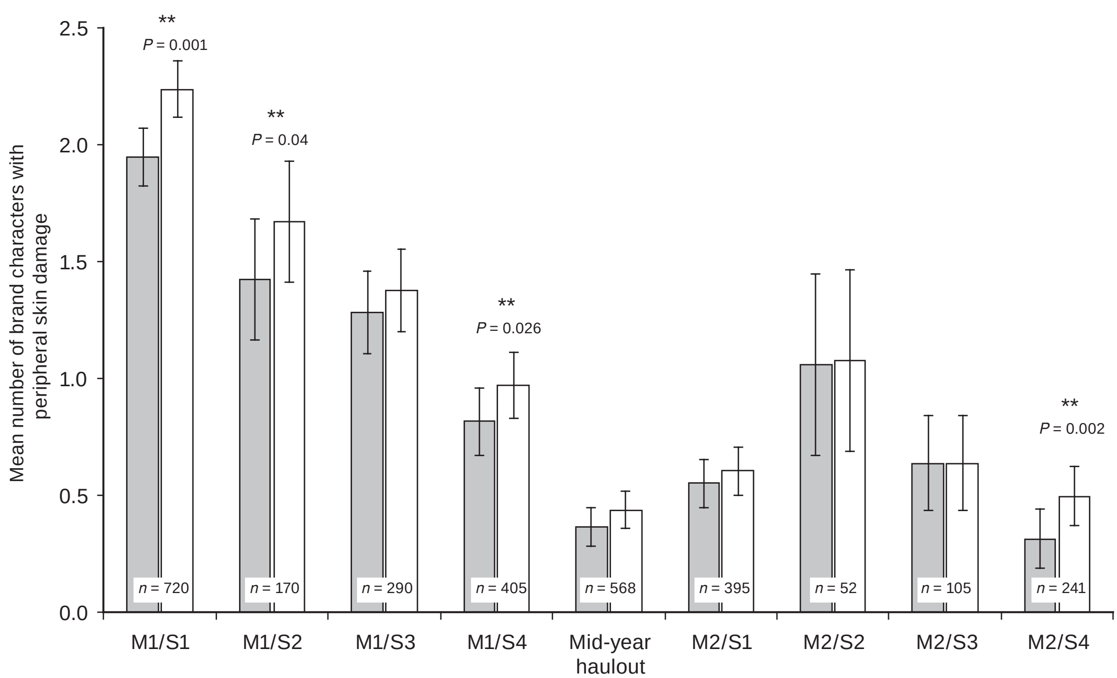

Fig. 4. Change in the mean number of brand characters with peripheral skin damage in the sample population of branded southern elephant seals observed during their first annual moult, mid-year haul-out when the seals are not moulting and the second moult. See Materials and Methods for descriptions of codes used.

good-quality brands or those with tags only (i.e. seals that were not branded).

The future brand quality was also significantly related to some of the physical and behavioural aspects associated with the branding event. Our findings point to some modifications to the branding event. We now know that because male weanlings are, on average, heavier than female weanlings (McMahon *et al*. 1997), the males require a much higher level of restraint than we exerted because more brand smudging (resulting from seal movement during branding) was found on heavier (stronger) and male pups. Weather conditions also influenced future brand quality because on rainy days, when pups were wet, more brands broke through the skin, and this we attribute to over-compensation in brand-application pressure for a perceived difference in wet versus dry seal fur, yet wet seals had fewer smudged brands. Branding wet seals with the appropriate pressure to avoid breaking the skin on a relatively flat level surface (preferably a sandy beach), and taking care to restrain larger (male) seals with extra effort was likely to produce a high-quality brand.

In a recent review of pinniped marking techniques, Erickson *et al*. (1993) discussed the advantages and limitations associated with temporary (e.g. dyes), semi-permanent (tags) and permanent (e.g. branding) marking techniques. Tagging, the most-widely used pinniped-marking technique, suffers from differential (often poor and unknown) tag-retention rates (Erickson *et al*. 1993), and tag breakage, tag colour fading and reduced symbol clarity over time (Broderick and Godley 1999). Hot-iron branding of seals can be controversial (McGilvray 2000) and highly emotive, yet our data show that in most cases (>95%) the method produced a healed and legible marking from which individuals could be identified with a high degree of confidence. After the initial stress of the capture and branding events is over (Australian Antarctic Division, unpublished data), the brands we applied are permanent markings that can be recorded with little or no further disturbance to the individual. Indeed, legible brands have been seen 23 years after branding (Hindell and Little 1988), while others have reported little or no disturbance to seals when attempting to identify branded seals in the field (Engelhard *et al*. 2001; Gales 2001).

For a marking procedure to be effective it should meet five essential criteria (Anon 1998): (1) the animal should experience no hindrance or irritation from the procedure, (2) the animal should suffer no adverse effects on its normal life behaviours, (3) the procedure should be quick, (4) the mark should be readily visible, and (5) the mark should be effective in allowing the research objectives to be met. We have shown that hot-iron branding meets these criteria for most southern elephant seals branded during this study at Macquarie Island. Also, many researchers in Alaska now use gas anaesthesia on Stellar's sea lions (*Eumetopias jubatus*) (Heath *et al*. 1996), which virtually eliminates Criteria 1 and 2. While we did not use anaesthesia, we have determined from another study (Australian Antarctic Division, unpublished data) that hot branding does not negatively affect survival nor do the brands

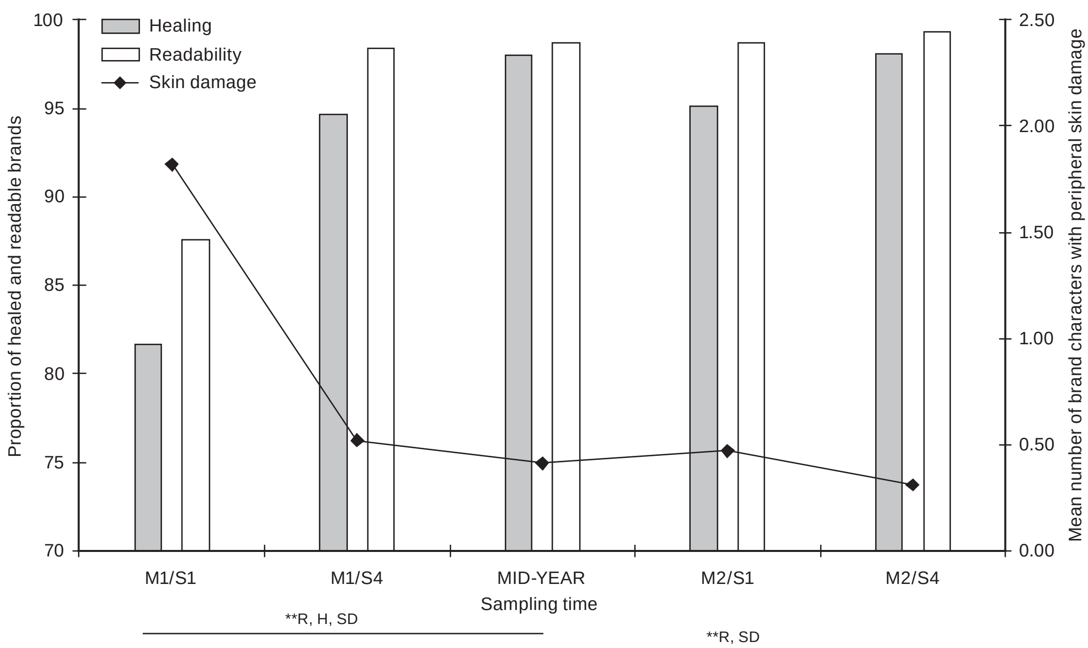

Fig. 5. Significant temporal changes in the quality of hot-iron brands on repeatedly measured southern elephant seals observed during their first annual moult, mid-year haul-out when the seals are not moulting and through to second moult. ** R, H, SD indicates that statistically significant changes occurred in the period marked by the underlying line. R = readability, H = healing, SD = skin damage.

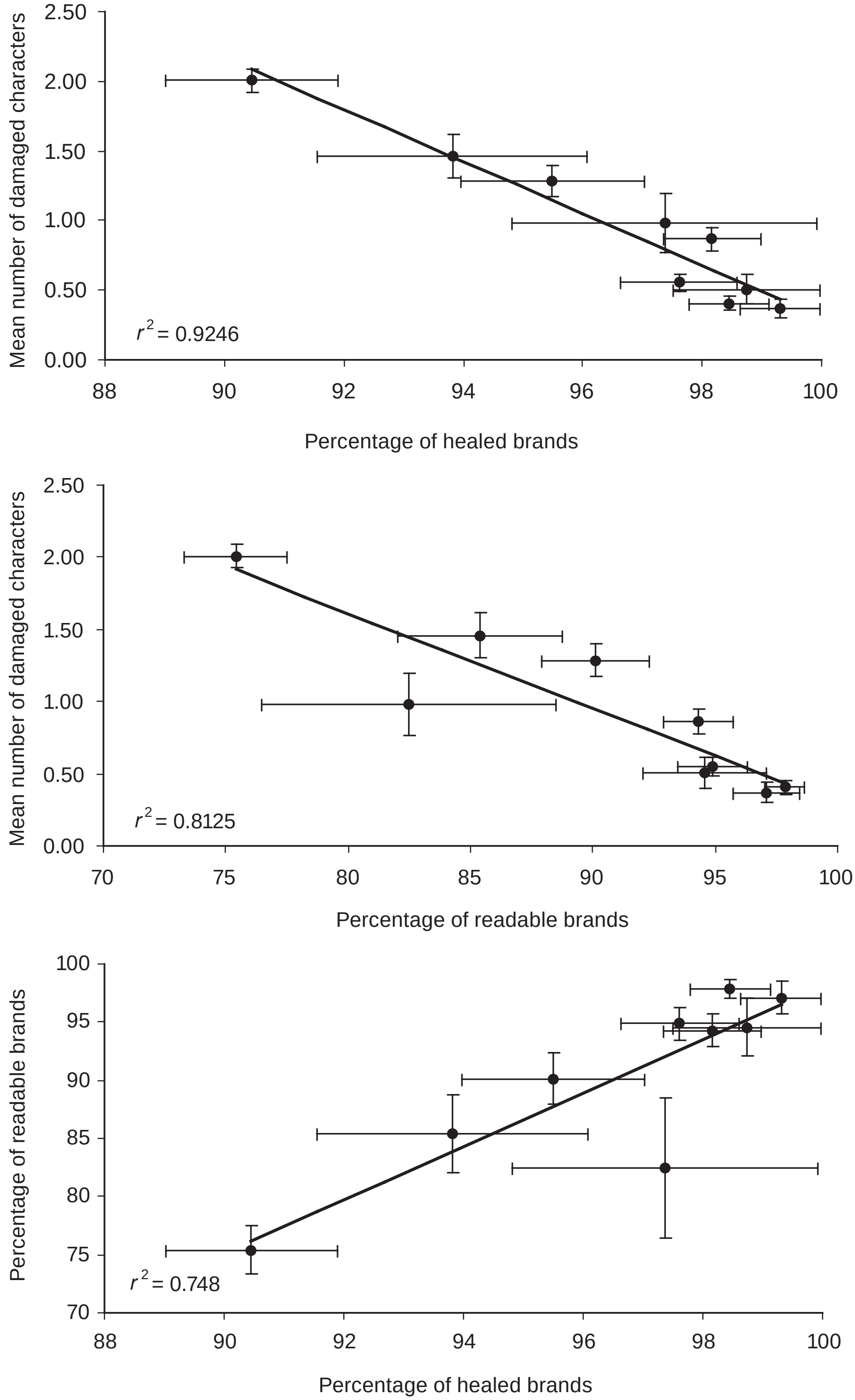

Fig. 6. Scatterplots of (*a*) the mean number of brand characters with peripheral skin damage and the percentage of healed brands; (*b*) the mean number of brand characters with peripheral skin damage and the percentage of readable brands; and (*c*) the percentage of readable brands and the percentage of healed brands. r^2 values are the correlation coefficients. The values indicate a strong to very strong linear correlation between the measured variables.

hinder the seal by catching and tearing from the flipper as a tag might. Branding was quick (3–4 s), depending on which side was branded, and the mark was readily visible from a distance for most of the seals; we have observed brands on older seals (7 year of age or more) that had increased in height to 200 mm without distortion and were very clearly visible even when the seals were concentrated in breeding harems. Thus, the objectives of a demographic or behavioural study would be met without compromising the study animal. When marking wild animals for research it must be ensured that the most appropriate marking method is used to meet the research objectives, while still remembering the criteria above. To this end we suggest that if hot-iron branding was again considered as a procedure for marking animals, a preliminary longitudinal study such as this one could be initiated to determine the longevity of hot-iron brand wounds and how brand identification changes over time.

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References

Anon. (1998). Live animal capture and handling guidelines for wild mammals, birds, amphibians and reptiles. (Eds M. McAdie, L. Westereng, and J. Quayle.) pp. 1–47. (British Columbia, Resources Inventory Committee.)

Ashwell-Erickson, S., Fay, F. H., and Elsner, R. (1986). Metabolic and hormonal correlates of molting and regeneration of pelage in Alaskan harbor and spotted seals (*Phoca vitulina* and *Phoca larga*). *Canadian Journal of Zoology* **64**, 1086–1094.

Bradshaw, C. J. A., Barker, R. J., and Davis, L. S. (2000). Modeling tag loss in New Zealand fur seal pups. *Journal of Agricultural Biological & Environmental Statistics* **5**, 124–134.

Broderick, A. C., and Godley, B. J. (1999). Effect of tagging marine turtles on nesting behaviour and reproductive success. *Animal Behaviour* **58**, 587–591. doi:10.1006/ANBE.1999.1183

Carrick, R., and Ingham, S. E. (1960). Ecological studies of the southern elephant seal *Mirounga leonina* (L.), at Macquarie Island and at Heard Island. *Mammalia* **24**, 325–342.

Carrick, R., Csordas, S. E., Ingham, S. E., and Keith, K. (1962). Studies on the southern elephant seal *Mirounga leonina* (L.). III. The annual cycle in relation to age and sex. *Wildlife Research* **7**, 119–160.

Caughley, G. (1977). 'Analysis of Vertebrate Populations.' (John Wiley and Sons: London.)

Chittleborough, R. G., and Ealey, E. H. M. (1951). Seal marking at Heard Island, 1949. ANARE Interim Report No. 1. (Ed. P. G. Law.) Department of External Affairs, Antarctic Division, Melbourne.

Csordas, S. (1995). An account of Australian seal marking studies in the Southern Ocean. *Aurora* **September**, 4–8.

Eberhardt, L. L., Chapman, D. G., and Gilbert, J. R. (1979). A review of marine mammal census methods. *Wildlife Monographs* **63**, 1–46.

Engelhard, G. H., van den Hoff, J., Broekman, M., Baarspul, A. N. J., Field, I., Burton, H. R., and Reijnders, P. J. H. (2001). Mass of weaned elephant seal pups in areas of low and high human presence. *Polar Biology* **24**, 244–251. doi:10.1007/S003000000204

Erickson, A. W., Bester, M., and Laws, R. M. (1993). Marking techniques. In 'Antarctic Seals: Research Methods and Techniques'. (Ed. R. M. Laws.) pp 89–115. (Cambridge University Press: Cambridge.)

Field, I. C., Bradshaw, C. J. A., McMahon, C. R., Harrington, J., and Burton, H. R. (2002). Effects of age, size and condition of elephant seals (*Mirounga leonina*) on their intravenous anaesthesia with tiletamine and zolazepam. *Veterinary Record* **151**, 235–240.

Fowler, J., Cohan, L., and Jarvis, P. (2000). How good are our estimates? In 'Practical Statistics for Field Biology'. 2nd edn. pp 90–103. (John Wiley and Sons: New York.)

Gales, N. (2001). A field review of the Macquarie Island elephant seal hot branding programme. Department of Conservation and Land Management, Perth. 27 pp.

Galimberti, F., Sanvito, S., and Boitani, L. (2000). Marking of southern elephant seals with passive integrated transponders. *Marine Mammal Science* **16**, 500–504.

Gentry, R. L., and Holt, J. R. (1982). Equipment and techniques for handling northern fur seals. NOAA Technical Report. NMFS SSRF-758.

Heath, R. B., Calkins, D. G., McAllister, D., Taylor, W., and Spraker, T. (1996). Telazol and isoflurane field anesthesia in free-ranging Steller's sea lions (*Eumetopias jubatus*). *Journal of Zoo and Wildlife Medicine* **27**, 35–43.

Hindell, M. A., and Burton, H. R. (1988). Seasonal haul-out patterns of the southern elephant seal (*Mirounga leonina* L.), at Macquarie Island. *Journal of Mammalogy* **69**, 81–88.

Hindell, M. A., and Little, G. J. (1988). Longevity, fertility and philopatry of two female southern elephant seals (*Mirounga leonina*) at Macquarie Island. *Marine Mammal Science* **4**, 168–171.

Krebs, C. J. (1989). 'Ecological Methodology.' (Harper Collins Publishers: New York.)

Ling, J. K. (1965). Hair growth and moulting in the southern elephant seal, *Mirounga leonina* (Linn.). In 'Biology of the Skin and Hair Growth'. (Eds A. G. Lyne and B. F. Short.) pp. 524–544. (Angus and Robertson: Sydney.)

McGilvray, G. (2000). Seal uproar: big questions arise. *Australian Veterinary Journal* **78**, 299.

McMahon, C. R., van den Hoff, J., Burton, H. R., and Davis, P. D. (1997). Evidence for precocious development in female pups of the southern elephant seal, *Mirounga leonina*, at Macquarie Island. In 'Marine Mammal Research in the Southern Hemisphere. Volume 1: Status, Ecology and Medicine'. (Eds M. Hindell and C. Kemper.) pp. 92–96. (Surrey Beatty: Sydney.)

McMahon, C. R., Burton, H. R., and Bester, M. N. (2000). Weaning mass and the future survival of juvenile southern elephant seals, Mirounga leonina, at Macquarie Island. *Antarctic Science* **12**, 149–153.

Merrick, R. L., Loughlin, T. R., and Calkins, D. G. (1996). Hot branding: a technique for long-term marking of pinnipeds. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-AFSC-68.

Scheffer, V. B. (1950). Experiments in the marking of seals and sea lions. U.S. Fisheries and Wildlife Service Special Scientific Reports No. 4. Washington, DC.

Troy, S., Middleton, D., and Phelan, J. (1997). On capture, anaesthesia and branding of adult male New Zealand fur seals *Arctocephalus forsteri*. In 'Marine Mammal Research in the Southern Hemisphere. Volume 1: Status, Ecology and Medicine'. (Eds M. Hindell and C. Kemper.) pp. 179–183. (Surrey Beatty: Sydney.)

Wilkinson, I. S., Padraig, D., and Childerhouse, S. (2001). An evaluation of hot-iron branding as a permanent marking method in the New Zealand sea lion, *Phocarctos hookeri*. In '14th Biennial Conference on the Biology of Marine Mammals, Vancouver, Canada, November 28–December 3'. (Society for Marine Mammals.)

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