

1 **Behavioral responses of juvenile Steller sea lions to hot-iron branding**

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13 **ABSTRACT**

14 Wildlife research frequently requires individual marking, such as hot-iron branding,
15 however procedures used may alter an animal's behaviors and cause pain. To date, studies have
16 assessed the immediate responses to hot-iron branding, however no study has assessed how hot-
17 iron branding affects the behavior of animals in the days following branding. Here we present the
18 first data showing the post-branding behavioral responses in a marine mammal. Eleven captive
19 juvenile Steller sea lions (*Eumetopias jubatus*) were observed for 3 days before and 3 days after
20 branding. Four of six monitored behaviors changed after branding. The proportion of time sea
21 lions spent in locomotion decreased from 0.07 (+0.02) before branding to 0.03 (+0.01) during the
22 first 24 h following branding (back-transformed mean + SE) and returned to baseline during the
23 second 24 h period. Wound-directed behavior (scratching, biting and head rubbing the branded
24 area) increased from 0.0 (+0.0) before branding to 0.01-0.02 (+0.01) during the first 48 h after
25 branding and returned to baseline thereafter. Time in the pool declined from 0.17 (+0.06) before
26 branding to 0.05 (+0.04) during the first 24 h after branding and returned to baseline by the
27 second 24 h period. The time spent with pressure on the branded side showed little change from
28 the 0.08 (+0.03) before branding to 0.10 (+0.03) during the first 24 h after branding; however,
29 this behavior decreased to 0.02 (+0.02) and 0.01 (+0.01) on following two days. These results
30 show that hot-iron branding affects Steller sea lion behavior up to 72 h. Changes in these
31 behaviours may be useful in assessing alternative effective analgesic protocols for this
32 procedure.

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34 Keywords: Behavior, *Eumetopias jubatus*, Hot-iron branding, Pain, Steller sea lions

35 **INTRODUCTION**

36 Animal conservation research often requires that individuals are marked and can thus be
37 followed over an extended period. One marking technique for marine mammals is hot-iron
38 branding. Brands provide researchers with a permanent and unique mark that can be used to
39 identify individual animals throughout their life [19]. Hot-iron branding has been used to identify
40 cattle and horses for centuries [14], and has been adapted for use in pinnipeds and non-
41 domesticated ungulates [19, 23].

42 Changes in physical appearance and behavior can be used as reliable indicators of pain
43 [22]. The immediate pain responses to hot-iron and freeze branding have been studied in cattle,
44 with hot-iron branding causing increased pain-related behaviors including tail flicking, kicking,
45 and vocalizations [26, 27] and greater escape-avoidance responses at the time of branding [11].
46 However, no published research has assessed an animal's behavioral responses in the days
47 following hot-iron branding.

48 Hot-iron branding of marine mammals has been criticized, resulting in a one-year
49 suspension of hot-iron branding of Steller sea lions in the USA [3] and an indefinite suspension
50 for elephant seals at Macquarie Island and Hooker's sea lions in New Zealand [2, 7, 16]. Despite
51 this concern, only four studies have been published that have examined the effects of branding in
52 pinnipeds, and all four studies focused only on physiological and survival effects. Steller sea
53 lions (*Eumetopias jubatus*) typically experience a general inflammatory response for up to two
54 weeks after branding [18]. In harbor seal (*Phoca vitulina*) pups, 76% of hot-iron brands were
55 found to have not healed at 9-10 weeks post-branding [4]. In Southern elephant seal (*Mirounga*
56 *leonina*) pups most brands healed within one year, but brands with more peripheral skin damage

57 had longer healing times [30]. Longer-term monitoring has found no difference in survival
58 between hot-iron branded and flipper-tagged Southern elephant seals [15].

59 The aim of our study was to describe the post-operative behavioral pain responses of
60 juvenile Steller sea lions following hot-iron branding. This research was conducted on sea lions
61 that were captured, brought into temporary captivity and underwent hot-iron branding, all as a
62 part of different research project [17]. Permitting constraints did not allow for experimental
63 treatment groups (e.g. with and without analgesics). We tested the hypotheses that: 1) the
64 behavioral responses measured before branding would differ from the 3 days following branding,
65 and 2) these differences would be most pronounced in the first 24 h period after branding.
66 Specifically, we hypothesized that animals would increase wound-directed behaviors, such as
67 rubbing and scratching the brand, and spend less time lying on the branded side.

68 **METHODS**

69 *Study design and animals*

70 Research was conducted in collaboration with the Alaska SeaLife Center (ASLC) in
71 Seward, AK, USA. All behavioral research conducted in this study was coordinated with the
72 ASLC's Transient Juvenile Steller Sea Lion Project [17] where an active hot-iron branding
73 program is already in place.

74 Eleven juvenile Steller sea lions, between 16 and 23 months of age, were captured in Prince
75 William Sound, AK, USA, in August 2007 (three males), February 2008 (one male and one
76 female) and August 2008 (four males and two females) under the Transient Juvenile Steller Sea
77 Lion Project, as described by [17]. Upon initial handling, symbols were shaved in the animal's
78 fur on their dorsal side to facilitate identification prior to hot-iron branding. The animals were
79 transported to the ASLC and held for up to 12 weeks prior to release.

80 The quarantine facility where the animals were held consisted of four adjoining pools (1 m x
81 4 m diameter and 3 m x 5 m diameter, 1.5 m deep), with unfiltered seawater from the directly
82 adjacent Resurrection Bay. Each pool was enclosed by 122 m² of metal surface haul-out area.
83 Animals could be housed individually or share access to multiple pools via sliding gates,
84 however animals were housed together to the maximum extent possible.

85 All hot-iron branding was performed for the ASLC's Transient Juvenile study, with no
86 additional handling required for our behavioral observations. No animals were branded simply
87 for the purposes of the current study. All research was conducted under Institutional Animal
88 Care and Use Committee protocols AUP07-009 (ASLC), A07-0342 (UBC), 08-26 (UAF) and
89 approved under NMFS permit #881-1890.

90 *Study procedures*

91 Sea lions were masked with 5% isoflurane in 100% oxygen for induction of anesthesia,
92 after which all animals were intubated. Anesthesia was maintained with 1% to 3% isoflurane in
93 100% oxygen delivered via a semiclosed, partially rebreathing circuit.

94 Hot-iron brand marks consisted of a combination of four numerals. Brands were applied
95 with specially designed stainless steel irons (each 10.2 cm high and 5.1 cm wide) that were
96 heated in a propane-fueled forge until the irons were cherry red. Each branding iron then was
97 applied to the left shoulder/flank area for 2 to 7 s each, as described by [18], with touch-ups to
98 each numeral lasting for 1 to 4 s each. Anesthesia ended when the branding was complete. The
99 average duration of anesthesia was 108.8 ± 16.4 min (mean \pm SE).

100 *Behavioral observations*

101 All behaviors were monitored by live focal animal observations for a total of 6 days: 3
102 days before branding and 3 days immediately following branding. Days were calculated as 24 h

103 periods, with Day 0 starting immediately following extubation from anesthesia. With the
104 exception of the day of branding, sampling occurred on all animals six times a day in 10 min
105 periods, twice during each period of the day (morning, 0900-1100 hours; afternoon, 1300-1500
106 hours; and evening, 1700-1900 hours). Behaviors were recorded using point-in-time sampling
107 (one scan every min for 10 min). On the day of branding, focal animals were observed after
108 recovering from anesthesia, which was 1.5 h after extubation from anesthesia (based on [9]).
109 During this initial observational period, sea lions were monitored continuously with behaviors
110 recorded every min for 1 h. After the first hour of observation, 10 min observations resumed.

111 All observations were by a trained observer was hidden from the sea lions' view behind a
112 plastic blind or via one-way glass depending upon the location of the focal animals. Reliability of
113 this observer's scores was estimated by comparing scores with another trained observer for 196
114 scans on six animals. The two observers showed 97% agreement for time spent alert and
115 complete agreement for all other behaviors scored.

116 We selected six behaviors related to post-operative pain using the following a priori
117 rationale: (a) Wound-directed behaviors: based on work in other species that have assessed
118 behavioural changes in the days following painful procedures (e.g. [20]), we predicted that sea
119 lions would increase wound-directed grooming and decrease the time spent with pressure on the
120 branded area during periods of lying and sitting; (b) Time spent in daily activities on land or
121 water: animals have been known to increase or decrease activities after painful procedures
122 depending on the use of the wounded area in performing that behavior. Based on work in other
123 species (e.g. [6]), we anticipated a reduction in time spent in locomotion and time spent in the
124 pool; (c) Time spent alert and attentive: animals exposed to noxious or painful stimuli increase
125 attentiveness or time spent alert [25]. Therefore, we predicted that sea lions would increase time

126 alert after hot-iron branding; and (d) Time spent lying down: based on work in other species after
127 painful procedures [10], we predicted that sea lions would decrease time lying after branding.

128 Mutually exclusive behaviors included locomotion, wound-directed grooming and lying
129 time. Locomotion was scored when the sea lion moved on land (i.e. all four limbs are moved at
130 least one body length either by walking on four flippers or by sliding its body across land with or
131 without its full belly on the surface) or in water (i.e. swimming - actively propelling itself
132 through the water at least one body length). Wound-directed grooming behaviors included
133 scratching, biting and head rubbing the branded area. Sea lions scratched themselves using the
134 front or rear flippers to scrape at their skin. Biting involved the use of teeth to grip or hold the
135 left side branded area, typically in a fast repetitive motion. Head rubbing involved moving the
136 head back and forth with pressure on skin surface. Sea lions were classified as lying down when
137 their bodies were flat on the ground, including dorsal, ventral, and left and right side positioning;
138 the head could be either lifted up or on the ground.

139 Non-mutually exclusive behaviors included time spent in the pool, time on the left
140 branded side and the time spent alert. Time spent in the pool was calculated from activities that
141 occur in the water (i.e. locomotion, floating and foraging). Time spent on the left branded side
142 was scored when an animal was lying or sitting on land with the majority of their body weight
143 and pressure on the left side of their body. Time spent alert was scored when an animal was
144 attentive with both eyes open.

145 To determine sample size required, power calculations were computed using preliminary
146 results from August 2007 data (Piface software Version 1.63). The analysis indicated that a
147 sample size of between five and 10 individuals would be required to accurately identify the
148 behavioral effects of hot-iron branding. Additionally, the sampling method used has been

149 previously found to accurately represent the proportion of time sea lions spend in each of the
150 behaviors [32].

151 *Statistical analysis*

152 The proportion of time sea lions spent displaying each behavior was averaged across the
153 3 days before branding (nominally Pre-brand) to generate one baseline measure per animal. For
154 each of the 3 days following surgery (nominally Day 0, Day 1 and Day 2), one measure per
155 animal was calculated to give the proportion of time each animal spent in each of the six
156 behaviors. Proportional data were outside the range of 0.3 to 0.7; therefore, to condense the
157 distribution and to allow for use in the parametric analyses, all data were arcsine square root
158 transformed ($Y = \arcsine \sqrt{p}$). Using a mixed model (SAS v9.1), with a compound symmetry
159 covariance structure, an analysis was conducted to test the effects of hot-iron branding on the six
160 behaviors. The model included the effect of day (Pre-brand, Day 0, Day 1 and Day 2). The
161 residuals from the models were tested against the basic assumptions of normality and variance
162 homogeneity, as well as plotted against the predicted values for the model. Three specified
163 contrasts were run to compare Pre-brand with each of the 3 days following hot-iron branding (i.e.
164 Pre-brand vs. Day 0, Pre-brand vs. Day 1 and Pre-brand vs. Day 2). In all cases, differences were
165 considered to be significant at $p \leq 0.05$. For the simplicity of interpretation, data in Fig. 1 and
166 Fig. 2 are presented back-transformed means with the positive SE.

167 **RESULTS**

168 Of the six behaviors measured, four changed after hot-iron branding; locomotion, wound-
169 directed grooming behaviors, time in the pool and time spent with pressure on the left (branded)
170 side.

171 The three mutually exclusive behaviors (i.e., locomotion, grooming the branded area and
172 lying time) are illustrated in Fig. 1. Time in locomotion on land and in water decreased from 7%
173 in the Pre-brand period to 4% in Day 0 ($F_{1,30} = 4.40, p = 0.044$), and returned to baseline by Day
174 1. Sea lions were rarely witnessed scratching, biting or rubbing their left side (area to be
175 branded) in the Pre-brand period. However in the days following hot-iron branding wound-
176 directed grooming increased, with sea lions occupying approximately 2% of their day on Day 0
177 and 1 after branding ($F_{1,30} = 10.02, p = 0.004$ and $F_{1,30} = 5.71, p = 0.023$, respectively) grooming
178 their left (branded) side. Time spent grooming the branded area returned to Pre-brand levels by
179 Day 2. For comparison, time spent grooming the right side showed no differences on Day 0, 1 or
180 2 when compared with the Pre-brand period ($F_{1,30} = 0.47, p = 0.50, F_{1,30} = 0.18, p = 0.67$ and $F_{1,30}$
181 $= 0.10, p = 0.76$, respectively). Similarly, grooming other areas (e.g. head, rump) did not increase
182 in Day 0, 1 or 2 when compared with the Pre-brand period ($F_{1,30} = 0.48, p = 0.50, F_{1,30} = 0.57, p$
183 $= 0.46$ and $F_{1,30} = 1.82, p = 0.19$, respectively). No differences were found in lying time.

184 The three non-mutually exclusive behaviors (i.e. time in the pool, time spent with
185 pressure on the left branded and time alert) are illustrated in Fig. 2. Prior to branding, sea lions
186 spent 17% of their time in the pool. Time in the pool declined to 5% on Day 0 ($F_{1,30} = 5.69, p =$
187 0.024), but returned to Pre-brand levels on Days 1 and 2. The time sea lions spent with pressure
188 on their left (branded) side showed little change from Pre-brand to Day 0 (N.S.), but decreased to
189 near zero on Day 1 and 2 ($F_{1,30} = 4.28, p = 0.047$ and $F_{1,30} = 7.01, p = 0.013$, respectively). No
190 differences were found in the time spent alert.

191 **DISCUSSION**

192 In the three days following hot-iron branding sea lions increased wound-directed
193 grooming and spent less time with pressure on their left branded side. Increased grooming

194 included head rubbing and scratching the branded area, similar to the wound-directed behaviors
195 witnessed after tail docking and dehorning in calves [5, 29, 31], and in decapods after exposure
196 to antenna-directed noxious stimuli [1].

197 Time spent with pressure on the branded side changed in the days following branding.
198 The deck surface was metal, so the increased time on the branded side on Day 0 might have
199 provided a cooling effect for the burn wound. The subsequent reduction in time on the branded
200 side on Days 1 and 2 may be due to increased sensitivity to pain, or hyperalgesia associated with
201 inflammation in the days after injury [13]. Burn injuries in animals that are not treated can lead
202 to sensitivity to previously innocuous stimuli (i.e. allodynia; [24]).

203 The time sea lions spent in activities on land and water decreased following hot-iron
204 branding. Specifically, sea lions decreased the time they spent in the pool and engaged in
205 locomotion during the first 24 h after branding; both behaviors returned to Pre-brand levels by 48
206 h after branding. Pain is also known to affect locomotion activity [6, 21]; decreased time in the
207 pool may also be associated with a sensitivity of the brand to salt water. Locomotion is often
208 decreased in human burn victims if the burn location is in an area that is highly mobile or
209 stretched during locomotion [8]. Sea lion recovering from abdominal surgery also showed
210 reduced locomotion for 3 days following surgery [32].

211 Comparing changes in behaviors before and immediately after hot-iron branding is a first
212 step in understanding pain responses. However, future studies should include branded and
213 unbranded animals, as well as analgesia and anaesthetic control groups (with no hot-iron
214 branding; [33]). Analgesia could involve pre-emptive administration of a local nerve block, the
215 use of oral or intra-muscular administration of analgesics, and the use of local anaesthetic gels
216 and sprays applied after branding. For example, administering a local anaesthetic to calves was

217 shown to reduce behavioral and physiological responses after dehorning [28]. Such studies
218 should also include an assessment of the logistics of the procedure for field use (where branding
219 typically occurs) where follow-up may be impossible.

220 Alternative methods of marking sea lions also warrant further investigation. It has been
221 suggested that cold-iron branding may cause less pain. While hot-iron brands burn through the
222 dermal layers and disrupt the hair follicles preventing new hair growth, cold branding damages
223 the pigment-producing melanocytes but leaves the hair follicles intact allowing for regenerative
224 growth of white hair [4, 23]. Studies on cattle show that cold-iron branding causes less of a pain
225 response than hot-iron branding [11, 12, 26]. For some pinniped species, however, it has been
226 shown that while cold-iron brands may heal faster, hot-iron brands are longer lasting and more
227 legible [4, 15].

228 In summary, in the three days following hot-iron branding Steller sea lions spent more
229 time grooming the branded area, less time with pressure on their left branded side, less time in
230 the pool and less time in locomotion. These behavioral responses may be useful in monitoring
231 pain following similar procedures in sea lions and other marine mammals and in developing
232 alternative pain management strategies for this procedure.

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330 **Figure 1.** Mutually exclusive behaviors: back-transformed means (+ SE) for the proportion of
331 time sea lions spent **(a)** in locomotion, **(b)** grooming the branded area and **(c)** lying down, before
332 and after hot-iron branding. Time series data are shown as Pre-brand (mean of 3 d before
333 branding), Day 0 (1st 24 h period after branding), Day 1 (2nd 24 h period after branding) and Day
334 2 (3rd 24 h period after branding). Significant differences for specified contrasts (Pre-brand vs.
335 Day 0, Day 1 and Day 2) are denoted by: * ($p \leq 0.05$) and ** ($p \leq 0.01$).

336 **Figure 2.** Non-mutually exclusive behaviors: back-transformed means (+ SE) for the proportion
337 of time sea lions spent **(a)** in the pool, **(b)** with pressure on their left (branded) side and **(c)** alert,
338 before and after hot-iron branding. Time series data are shown as Pre-brand (mean of 3 d before
339 branding), Day 0 (1st 24 h period after branding), Day 1 (2nd 24 h period after branding) and Day
340 2 (3rd 24 h period after branding). Significant differences for specified contrasts (Pre-brand vs.
341 Day 0, Day 1 and Day 2) are denoted by: * ($p \leq 0.05$) and ** ($p \leq 0.01$).



