

**MAKING OUR MARK:
ASSESSMENT OF THE BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF LONG
TERM TRACKING METHODS IN STELLER SEA LIONS (*EUMETOPIAS JUBATUS*)**

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are needed to see this picture.

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ABSTRACT

Marine mammal research often requires marking animals in order to collect important long-term ecological data. However, there is increasing scrutiny of both well-established and new techniques, such that careful assessment of the impact of the procedure itself on the individual is of paramount importance. We examined behavioral and physiological responses of endangered juvenile Steller sea lions (*Eumetopias jubatus*) to hot-iron branding and surgical implantation of Life History Transmitters (LHX). Specifically, key behaviors were observed prior to and after each procedure, paired with physiological indicators of immune response. Assessments of post-LHX recovery identified 2 distinct behaviors (standing, back arch) that may function as indicators of surgical pain responses. This suggests that the post-operative analgesia could be modified to provide additional mitigation. Hot-iron branding resulted in decreased locomotion, increased wound-directed behaviors, decreased time spent on the left, branded side and a decreased time spent in the pool. Three of the four behaviors returned to baseline within the 72hr observation period, however time spent with pressure on the branded side was still decreased at 72hr post-branding. Physiological changes were noted with both procedures, however they lie within previously published range for sea lions.

INTRODUCTION

Marine mammals are apex predators that have major economic impacts on many fisheries areas, including the Bering Sea. The dramatic decline of several species of marine mammals in this particular region has prompted much research into both the general biology, potential causes of the decline, and factors surrounding the work itself. We proposed to address a fundamental issue relevant to **PCCRC Research Priority #1 – Factors influencing the sustainability of marine mammal populations**. Sustainability can only be modeled accurately with population assessments. For Steller sea lions (*Eumetopias jubatus*), population estimates are derived through aerial surveys (Fritz and Stinchcomb 2005; Angliss and Outlaw 2007), mark-recapture methods (Merrick *et al.* 1994; Merrick and Loughlin 1997; Loughlin *et al.* 2003; Raum-Suryan *et al.* 2004) or long-term tracking of individuals (Merrick *et al.* 1996; Horning and Hill 2005). Aerial surveys are low-disturbance but have an inherently potentially large margin of error. In contrast, temporary or permanent marks may provide more accurate information, but are often viewed as controversial. Concerns specific to hot-iron branding were included in part of a successful lawsuit brought against the National Marine Fisheries Service by the Humane Society of the United States (2006). In large part, the impact of this and other permanent marking measures on the individual had not been examined in detail. We studied the behavioral and physiological effects of two currently utilized protocols in Steller sea lion population monitoring: 1) hot-iron branding and 2) abdominal implantation of Life History Transmitters (LHX tags). Well known for its use in domestic farming practices, hot-iron branding has been an accepted method for decades (Macpherson and Penner 1967). However, the application of this method to pinnipeds (e.g., Merrick *et al.* 1996) allows for collection of life history data, movement patterns, and site fidelity data (NMFS 2002), but the effects of the method on the individual are just beginning to be examined (Daoust *et al.* 2006; McMahon *et al.* 2006; Mellish *et al.* 2007b). Alternatives to external markings for long-term survival data are also being developed, including the LHX tag. This abdominally implanted device collects life-long data on dive behavior, pressure, motion, light levels, temperature and conductivity (Horning and Hill 2005). As with hot-iron branding, efforts to understand the impact of the procedure are critical (Mellish *et al.* 2007a).

OBJECTIVES

The specific objectives of this proposal were to:

1. Conduct behavioral observations in order to identify key behaviors exhibited during and after two types of invasive, long-term marking/monitoring procedures (i.e., hot-branding and LHX implantation).
2. Compare key behaviors with physiological parameters of inflammation (e.g., elevated leukocyte count, acute phase response).
3. Develop objective pain assessment methods for sea lions and apply these methods in identifying and reducing pain during invasive marking procedures

STUDY AREA

All observations and samples were collected at the Transient Juvenile Steller Sea Lion Facility at the Alaska SeaLife Center, Seward. This is a specialized quarantine environment dedicated to the study of juvenile Steller sea lions (Mellish *et al.* 2006). All sea lions were collected in groups of four to six individuals from juvenile-only haul-out locations in Prince William Sound and Resurrection Bay. Sea lions were released after ten to twelve weeks of temporary captivity for research purposes.

EXPERIMENTAL METHODS

Upon arrival to the ALSC, animals were randomly assigned to one of two treatment groups for each marking method study. To facilitate individual identification prior to hot-iron branding, all sea lions were given unique numbers shaved in their fur on their dorsal side. Animals were housed together in four adjoining outdoor pools unless separation was required for husbandry or handling procedures. Baseline observations occurred for 3 days before any marking procedure (LHX implant or branding). A total of nine sea lions were included in the LHX study (n=5, August 2007; n=4, February 2008). To study the cumulative effects of the procedures, five of the sea lions received both an LHX implant and a hot-iron brand on the same day (Group 1). The remaining four sea lions received only an LHX implant followed by a hot-iron brand 2 weeks later (Group 2). All animals were under general anesthesia (isoflurane gas) at the time of the

LHX implant and received a post-operative analgesic (flunixin). Eleven sea lions, from captures in August 2007, February 2008 and August 2008, were included in the branding study. All animals were under general gas inhalant anesthesia (isoflurane) at the time of hot-iron branding.

For both studies, all behaviors were monitored for 7 days (3 days before a procedure, day of procedure, to 3 days following a procedure). To evaluate when the behavioral responses began to diminish in the LHX study, animals were also observed in days 12-15 post-surgery. Behaviors were monitored during live observations during 3 day-parts (morning 9:00-11:00, afternoon 13:00-15:00, and evening 17:00-19:00). Focal animal sampling occurred on all animals six times a day, twice during each day part. On procedure days, focal animals were observed once pre-procedure, for 1 hr post procedure (which took place 1 hr after anesthesia while being held in a dry run between pools), and for the first post-procedure access to food, pools, and other sea lions. Point in time sampling was used to record behaviors (one sample every minute for ten minutes). Behaviors included social interactions (e.g., wrestle, bite, chase), positional behaviors (e.g., float, sit upright, prone, alert), proximity to others, feeding behaviors, as well as vocalizations and specific wound directed behaviors. All occurrence sampling was used to monitor total time spent in the water. Data validation and power analysis selected six behaviors to measure the behavioral responses to hot-iron branding, including time spent on the left side, locomotion, time spent alert, grooming behaviors, time spent in the pool and time spent lying down. Similarly, key behavioral responses identified to monitor response to LHX implant surgery included sit up, lie down, stand, back arch, locomotion and time spent alert, as well as lying position (right, left, dorsal or ventral).

Physiological parameters of inflammation and healing were assessed during routine health assessments at a minimum of two weeks prior to the implant/branding event, the day of the event, and two weeks post-event, as set by the handling guidelines for the larger Transient Project (MMPA 881-1890). These parameters included, but were not limited to, white blood cell count, platelets, hematocrit, fibrinogen and globulins. Methods for these analyses are documented in detail in Mellish *et al.* 2006 and Thomson and Mellish 2007.

SUMMARY OF RESULTS

LHX- The manuscript from this portion of the experiment, '*Behavioural responses of juvenile Steller sea lions to abdominal surgery: Developing an assessment of post-operative pain*' has been published in Applied Animal Behaviour Science (Walker *et al.* 2009, attached). In summary, six of the seven behaviors were found to change after LHX surgery (**TABLE 1**). In particular, there was an effect of day for two behaviors that were rarely observed prior to surgery: standing and back arching. These behaviors were still present at 10-12 post-surgery.

Hot-iron branding- Data collection (behavioral and physiological) was completed for this portion of the project in late September 2008. Fibrinogen samples have been processed however the data analysis for these samples is pending. A manuscript currently under review in the journal Pain (Walker, Mellish and Weary), entitled '*Behavioural responses of juvenile Steller sea lions to hot-iron branding*' describes the findings of this study in detail. In summary, four of six monitored behaviors changed after branding (**FIGURES 1 & 2**). In the three days following branding, sea lions spent more time grooming the branded area, less time with pressure on their branded side (left), less time in the pool and in locomotion. All behaviors except time spent with pressure on the branded side returned to baseline by 72hr post-branding.

DISCUSSION

In the LHX portion of this study, we found that standing, back arching, and lying time increased, and time spent resting on the ventral surface, time alert, and overall locomotion on land and water decreased in the days following abdominal surgery. Standing and back arching were not witnessed prior to surgery. Sea lions may perform these behaviors to promote the recovery of the wound and reduce stimulating the area of the injury. An increased sensitivity to pain, or hyperalgesia, is known to occur in response to inflammatory pain and may explain why we see the sea lions back arching and standing at 2 weeks post-surgery.

LHX implant surgery resulted in an expected elevation in white blood cells, peaking at 2 weeks post-surgery, with levels returning to capture levels prior to release. White blood cell increases may be witnessed during general stress responses, as well as in response to physical insults such as laceration or abscesses to varying degrees. The elevation witnessed in this study due to LHX

surgery lies within the previously recorded ranges for Steller sea lions, and is significantly lower than counts witnessed in sea lions with abscesses (Mellish *et al.* 2006; Mellish *et al.* 2007a, Mellish *et al.* 2007b). While the current study design does not allow for a long-term assessment of the procedure, we show that the distinct physiological impact is short-lived.

In the hot-iron branding portion of the study, we found that sea lions increased wound-directed grooming and decreased the time spent in the pool and in locomotion, however, these responses returned to baseline by 72hr post-branding. Sea lions also spent less time with pressure on their left branded side, although this response did not diminish by 72hr post-branding. This may be due to sea lions experiencing an increased sensitivity to pain associated with inflammation in the days after injury. The behavioral responses to hot-iron branding suggest that pain management strategies such as pre-operative administration of analgesia or local anesthetics should be investigated.

CONCLUSIONS

Wildlife research often requires that researchers mark animals so individuals can be followed over an extended period. While such procedures may alter an animal's natural behaviors and may cause pain, no study to-date has evaluated pain responses in marine mammals. Our research is the first to describe distinct post-procedural changes in behavior, likely related to pain. These responses may be useful in monitoring pain following similar procedures in sea lions and other marine mammals. Physiological changes measured in this study were within the normal published ranges for sea lions and these responses receded prior to release at 4 weeks after surgery. Our results indicate that sea lions do respond behaviorally to invasive procedures, but some responses are mediated in a matter of days. Alternative pain management strategies such as the use of alternative analgesia or the administration of analgesics or local anesthetics pre-operatively merit further investigation to more comprehensively mitigate possible pain associated with abdominal surgical procedures and hot-iron branding.

ACKNOWLEDGEMENTS

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Jamie Thomson, Jeanette Nienaber, Pam Tuomi, Brett Long, Christy Phillips and Lisa Hartman. Markus Horning, Dan Weary and David Fraser contributed to the academic portion of the project. All procedures were carried out under National Marine Fisheries Service Marine Mammal Protection Act and Endangered Species Act permit #881-1890, University of Alaska Fairbanks Animal Use Protocol #08-26, Alaska SeaLife Center Animal Use Protocols # 07-009, 09-001, and University of British Columbia Animal Care Certificate #A07-0342.

REFERENCES

- Angliss, R. P. and R. B., Outlaw. 2007. Alaska marine mammal stock assessments, 2006. U.S. Department of Commerce, NOAA Tech. Memo. NMFSAFSC-168: 244 pp.
- Dalton, R. 2005. Animal-rights group sues over 'disturbing' work on sea lions. *Nature* 436: 315.
- Daoust, P., G.M. Fowler and W.T. Stobo. 2006. Comparison of the healing process in hot and cold brands applied to harbour seal pups (*Phoca vitulina*). *Wildlife Research* 33: 361-372.
- Fritz, L. W. and C. Stinchcomb. 2005. Aerial, ship and land-based surveys of Steller sea lions (*Eumetopias jubatus*) in the western stock in Alaska, June and July 2003 and 2004. U. S. Dep. Commer., NOAA Tech. Memo. NMFS-AFSC-153: 56 pp.
- Horning, M. and R.D. Hill. 2005. Designing an archival satellite transmitter for life-long deployments on oceanic vertebrates: the life history transmitter. *IEEE Journal of Oceanic Engineering* 30: 807-817.
- Lay, D.C., T.H. Friend, C.L. Bowers, K.K. Grissom and O.C. Jenkins. 1992a. A comparative physiological and behavioral study of freeze and hot-iron branding using dairy cows. *Journal of Animal Science* 70: 1121-1125.
- Lay, D.C., T.H. Friend, R.D. Randelt, C.L. Bowers, K.K. Grissom and O.C. Jenkins. 1992b. Behavioral and physiological effects of freeze or hot-iron branding on crossbred cattle. *Journal of Animal Science* 70: 330-336.
- Loughlin, T. R., J. Sterling, R.L. Merrick, J.L. Sease and A.E. York. 2003. Diving behavior of immature Steller sea lions. *Fisheries Bulletin* 101: 566-582.
- Macpherson, J.W. and P. Penner. 1967. Animal identification I. Liquid nitrogen branding of cattle. *Canadian Journal of Comparative Medicine and Veterinary Science* 31: 271-274.

- McMahon, C.R., H.R. Burton, J. VanDenHoff, R. Woods, and C.J. Bradshaw. 2006. Assessing hot-iron and cryo-branding for permanently marking southern elephant seals. *Journal of Wildlife Management* 70: 1484-1489.
- Mellish, J., D. Calkins, D. Christen, M. Horning, L. Rea and S. Atkinson. 2006. Temporary captivity as a research tool: comprehensive study of wild pinnipeds under controlled conditions. *Aquatic Mammals* 32: 58-65.
- Mellish, J., J. Thomson, and M. Horning. 2007a. Physiological and behavioral response to intra-abdominal transmitter implantation in Steller sea lions. *Journal of Experimental Marine Biology and Ecology* 351: 283-293.
- Mellish, J., D. Hennen, J. Thomson, L. Petrauskas, S. Atkinson and D. Calkins. 2007b. Permanent marking in an endangered species: physiological response to hot branding in Steller sea lions (*Eumetopias jubatus*). *Wildlife Research* 34: 1-6.
- Merrick, R. L. and T.R. Loughlin. 1997. Foraging behavior of adult female and young-of-the year Steller sea lions in Alaskan waters. *Canadian Journal of Zoology* 75: 776-786.
- Merrick, R.L., T.R. Loughlin and D.G. Calkins. 1996. Hot Branding: A technique for long-term marking of pinnipeds. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-AFSC-68.
- Merrick, R.L., T.R. Loughlin, G.A. Antonelis and R. Hill. 1994. Use of satellite-linked telemetry to study Steller sea lion and northern fur seal foraging. *Polar Research* 13: 105-114.
- Nietfeld, M.T., M.W. Barrett and N. Silvy. 1994. Wildlife marking techniques. Pages 140-168 in Research and management techniques for wildlife and habitats, Fifth ed.; Bookhout, T.A. (Ed.). The Wildlife Society.
- NMFS. 2002. Environmental assessment of the effects of NMFS permitted scientific research activities on threatened and endangered Steller sea lions. Office of Protected Resources, National Marine Fisheries Service, Silver Springs, MD.
- Raum-Suryan, K.L., M.J. Rehberg, G.W. Pendleton, K.W. Pitcher and T.S. Gelatt. 2004. Development of dispersal, movement patterns, and haul-out use by pup and juvenile Steller sea lions (*Eumetopias jubatus*) in Alaska. *Marine Mammal Science* 20: 823-850.
- Schwartzkopf-Genswein, K.S., J.M. Stookey and R. Welford. 1997a. Behavior of cattle during hot-iron and freeze branding and the effects on subsequent handling ease. *Journal of Animal Science* 75:2064-2072.

- Schwartzkopf-Genswein, K.S., J.M. Stookey, A.M. dePassille and J. Rushen. 1997b. Comparison of hot-iron and freeze branding on cortisol levels and pain sensitivity in beef cattle. *Canadian Journal of Animal Science* 77: 369-374.
- Schwartzkopf-Genswein, K.S., J.M. Stookey, T.G. Crowe and B.M.A. Genswein. 1998. Comparison of image analysis, exertion force, and behavior measurements for use in the assessment of beef cattle responses to hot-iron and freeze branding. *Journal of Animal Science* 76: 972-979.
- Thomton, J.D., and J.E. Mellish. 2007. Haptoglobin concentrations in free-range and temporarily captive juvenile Steller sea lions. *Journal of Wildlife Diseases* 43: 258-261.

TABLE 1. Least square means and S.E.M. for the proportion of time sea lions ($n = 9$) spent displaying behaviors before and after LHX implant surgery. Means and S.E.M. are the arcsine square root transformed values. Backtransformed means are provided in parentheses. Specified contrasts pre- vs. post-surgery and pre- vs. late post-surgery P-values are presented and considered significant at $P \leq 0.05$. *Results from Walker et al. (2009).*

| | pre-surgery | post-surgery | late post-surgery | S.E.M. | pre- vs. post-surgery P-value | pre- vs. late post-surgery P-value |
|----------------------------|-------------|--------------|-------------------|--------|-------------------------------|------------------------------------|
| Land and water behaviours: | | | | | | |
| Alert | 0.95 (0.66) | 0.85 (0.56) | 0.89 (0.60) | 0.04 | 0.02 | 0.20 |
| Locomotion | 0.23 (0.05) | 0.12 (0.01) | 0.25 (0.06) | 0.03 | 0.05 | 0.68 |
| Lying down | 0.71 (0.42) | 0.93 (0.64) | 0.96 (0.67) | 0.08 | 0.07 | 0.03 |
| on ventral side | 1.56 (1.0) | 0.43 (0.17) | 0.46 (0.20) | 0.06 | < 0.001 | < 0.001 |
| Stand | 0.00 (0.00) | 0.26 (0.07) | 0.20 (0.04) | 0.03 | < 0.001 | 0.001 |
| Back arch | 0.11 (0.01) | 0.86 (0.57) | 0.61 (0.33) | 0.06 | < 0.001 | < 0.001 |
| Water only behaviours: | | | | | | |
| time spent in pool | 0.51 (0.24) | 0.44 (0.18) | 0.45 (0.19) | 0.1 | 0.51 | 0.49 |

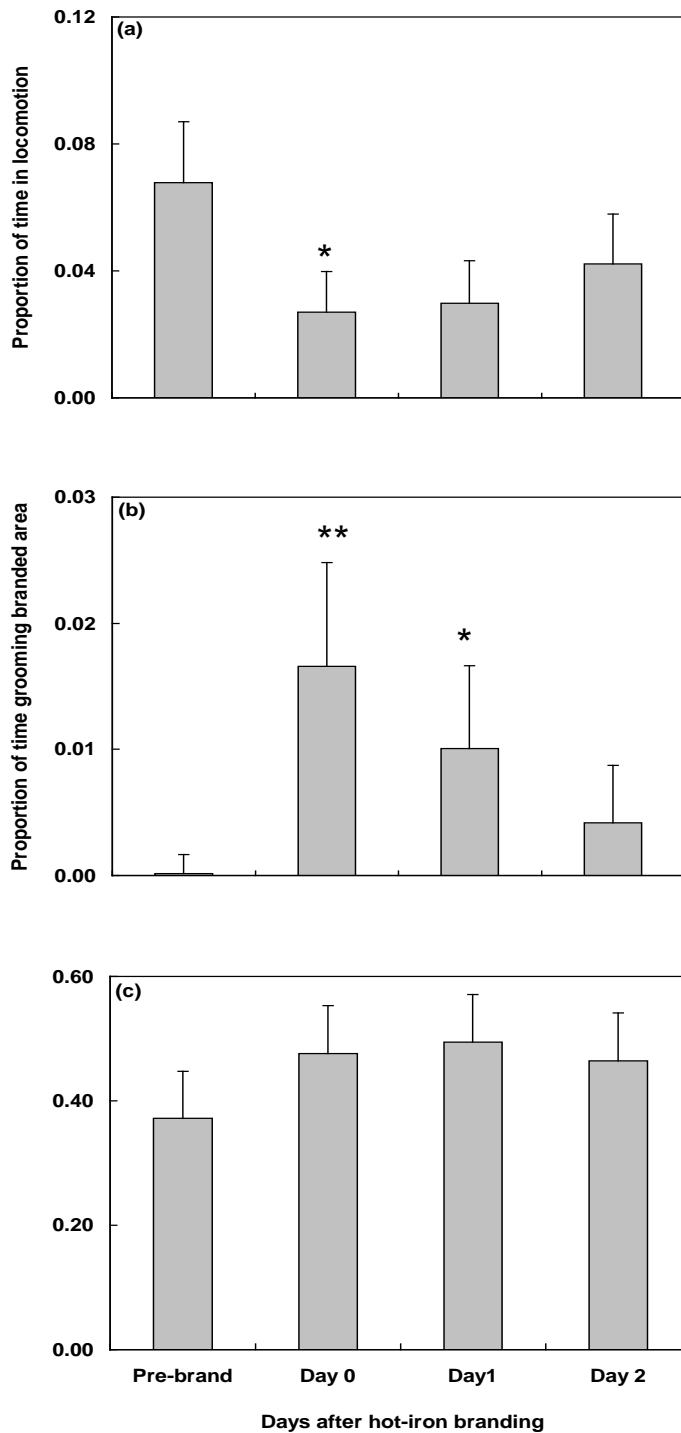


Figure 1. Mutually exclusive behaviors: back-transformed means (+ SE) for the proportion of time sea lions spent (a) in locomotion, (b) grooming the branded area and (c) lying down, before and after hot-iron branding. Time series data are shown as Pre-brand (mean of 3 d before branding), Day 0 (1st 24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

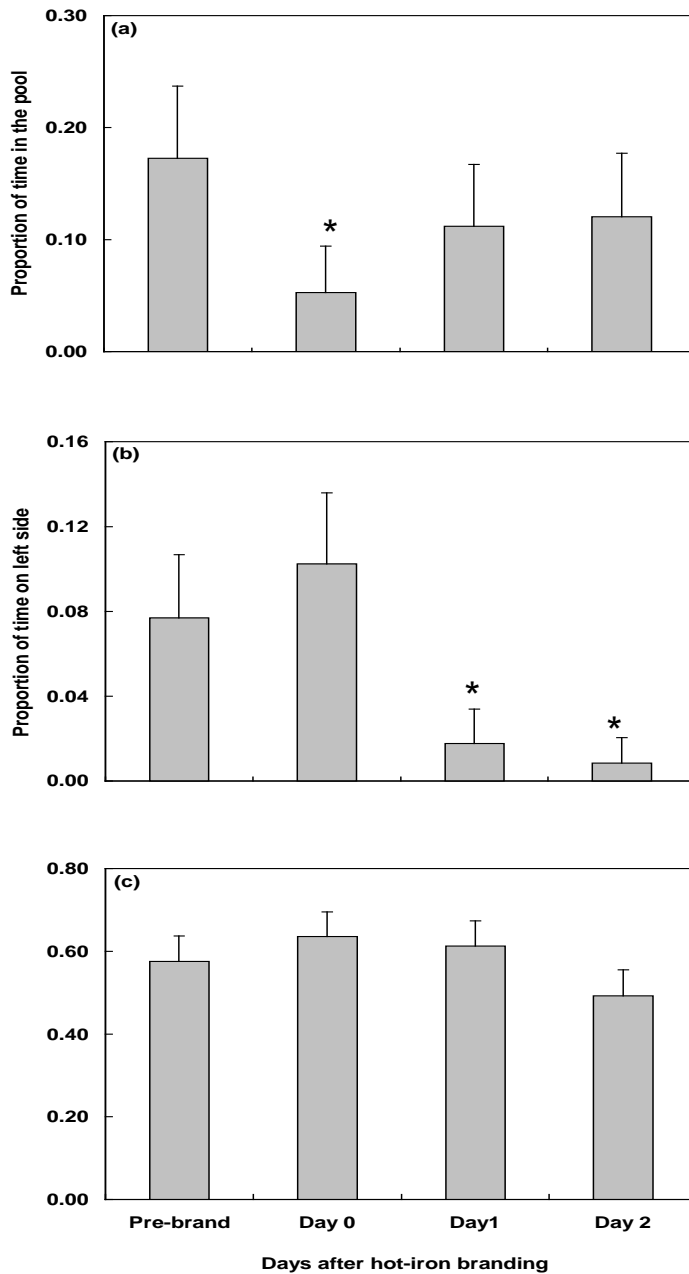


FIGURE 2. Non-mutually exclusive behaviors: back-transformed means (+ SE) for the proportion of time sea lions spent **(a)** in the pool, **(b)** with pressure on their left (branded) side and **(c)** alert, before and after hot-iron branding. Time series data are shown as as Pre-brand (mean of 3 d before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

STUDY PRODUCTS

This work will comprise a large portion of the Doctoral dissertation of K. Walker, University of British Columbia, in partnership with J. Mellish, University of Alaska Fairbanks and the Alaska SeaLife Center. K. Walker presented the results of this work at the annual meeting in Anchorage, 2009. Products of this funding include the two attached completed manuscripts (Walker et al., in press; Walker, Mellish & Weary, in review). The findings in these two manuscripts provide the foundation for an additional three manuscripts in preparation.

COMPLETED TIMELINE

August-October 2007: Pilot study data collection branding study (n=3), LHX study (n=5).

February 2008: PCCRC awarded.

February-April 2008: Detailed behavioral and physiological data collection for branding study (n=2) and LHX (n=4). Data collection for LHX portion completed.

May-July 2008: Data entry and preliminary statistical analyses.

August 2008: Travel (K. Walker & J. Mellish) to Vancouver, BC, for consultation with animal welfare experts, D. Weary and D. Fraser.

August-November 2008: Sea lion collection, with final individuals for post-branding study (n=6). Data collection and analysis continues for post-brand study.

November-December 2008: Completed data collection and data entry for both branding and LHX portions of the study. Preparation of post-branding study manuscript.

January 2009: Presentation by K. Walker to PCCRC annual meeting in Anchorage.

February 2009: Completion of all data analyses and submission of LHX manuscript.

May 2009: Travel (K. Walker) to Seward, AK, for preparation of hot-iron branding manuscript. LHX manuscript accepted for publication in Applied Animal Behaviour Science.

September 2009: Submission of hot-iron branding manuscript to journal Pain. Preparation of Final Report to PCCRC.

FINAL BUDGET

| Category | Item | Original | Rebudget | Final | Details |
|---------------------|------------------------|-----------------|-----------------|-----------------|---|
| Travel | | \$7,105 | \$6,974 | \$6,911 | 6 trips completed; JM (1) and KW (5) |
| Contractual | Housing | \$7,200 | \$10,101 | \$10,101 | 12 months, Seward KW |
| | Fibrinogen | \$720 | \$0 | \$333 | partially supplied by alternative funding |
| | Shipping | \$250 | \$0 | \$0 | alternative funding |
| | Publications | \$750 | \$300 | \$0 | none required for journals chosen |
| Commodities | Fibrinogen supplies | \$450 | \$0 | \$27 | alternative funding |
| | Misc | \$750 | \$400 | \$387 | project supplies |
| | Video multiplexer | \$250 | \$0 | \$0 | borrowed unit |
| | Audio recorder | \$300 | \$0 | \$0 | trials demonstrated recordings were not accurate in this environment |
| Total Budget | | \$17,775 | \$17,775 | \$17,759 | |