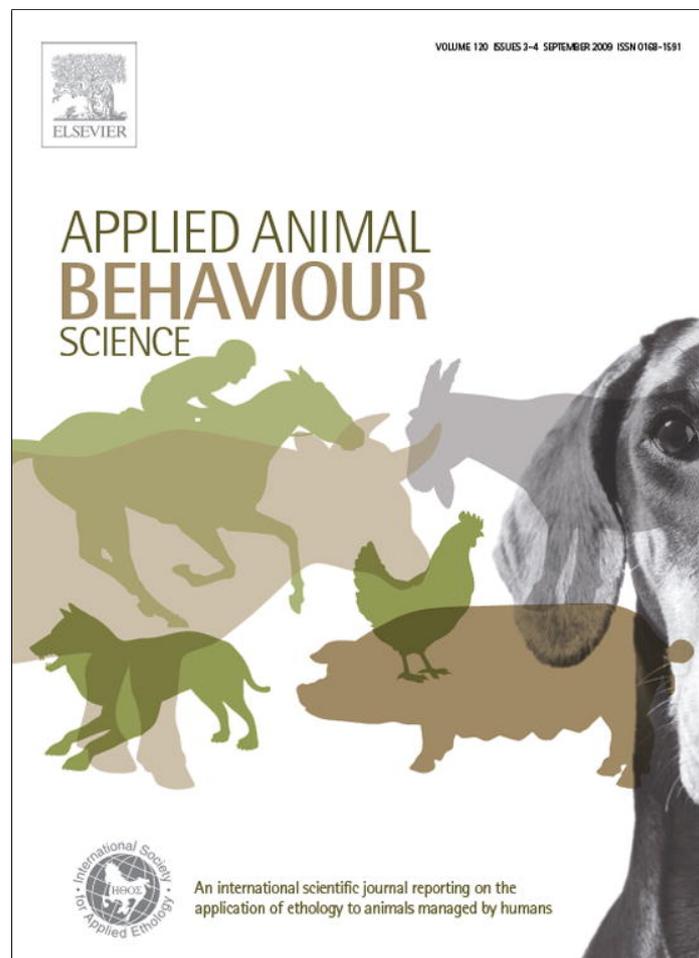


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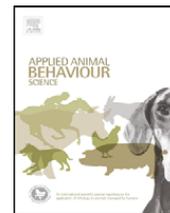
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Behavioural responses of juvenile Steller sea lions to abdominal surgery: Developing an assessment of post-operative pain

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ABSTRACT

Marking and tracking of marine mammals is required to gain a better understanding of life history traits; however, some marking procedures used are likely painful. Recent technological advances include intra-abdominally implanted archival telemetry devices for the life-long monitoring of individual animals. No research to date has assessed any aspect of post-operative pain in marine mammals. This study specifically evaluated behavioural responses in nine juvenile Steller sea lions to the abdominal surgery required for insertion of telemetry devices. Behaviours predicted to reflect post-operative pain, including posture and body movements, were assessed during 3-day pre-, 3-day post-, and days 10–12 post-surgery. The proportion of time sea lions spent on land standing increased from 0.00 to 0.07 and then decreased to 0.04, for pre-, post-, and late post-surgery respectively. Similarly, the proportion of land time spent with the back arched increased from 0.01 to 0.57, and then decreased to 0.33. The time sea lions spent on land with pressure on their ventral side while sitting or lying down declined from 1.0 pre-surgery to 0.17 post-surgery, and increased to 0.20 late post-surgery. The time sea lions spent in locomotion on land and in the water decreased from 0.05 in pre-surgery to 0.01 post-surgery, and returned to 0.06 by late post-surgery. These results suggest that behaviours such as back arch, standing, time spent with pressure on the ventral side, and locomotion may be useful in the assessment of pain following abdominal surgery in sea lions. The presence of these behaviours and their persistence for up to 12 days after surgery suggest that more work is required to further develop safe and effective analgesic methods for this procedure.

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1. Introduction

Considerable research has focused on painful procedures on farm animals, such as dehorning in dairy calves and castration in piglets (e.g. Weary et al., 2006); however, much less is known about pain responses in wildlife. Research on wildlife often requires the application of

marking and tracking devices (Murray and Fuller, 2000) and such procedures may cause pain. For example, marine mammals are sometimes marked using hot-iron branding and followed using tracking devices that are implanted via abdominal surgery.

Given the logistical difficulties of many marine mammal field studies, the opportunity to assess marking procedures has been limited or non-existent. Only recently have efforts been made to assess effects of some marking methods used (e.g. Daoust et al., 2006; Mellish et al., 2007a, b), and no study has experimentally addressed post-operative pain. In

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the absence of validated methods for pain assessment and treatment, some researchers may not provide, or fail to report the use of, analgesics following procedures such as hot- and cold-iron branding (van den Hoff et al., 2004; Daoust et al., 2006) and surgical implantation of radio transmitters (Ralls et al., 1989).

Several marine mammal species have experienced significant population declines over the past few decades, including the endangered Western population of Steller sea lions (*Eumetopias jubatus*). Long-term ecological data are essential for an understanding of past and present population trajectories, which requires animals to be individually identified (e.g. hot-iron branded, flipper-tagged) and monitored over long periods of time. Despite the controversy around some of these procedures (Green and Bradshaw, 2004; Dalton, 2005), and repeated calls for studies on the effects of marking (Murray and Fuller, 2000; Beausoleil and Mellor, 2007), there has been little research on the effects of marking of marine mammals including post-operative pain.

1.1. Pain assessment

The experience of pain is subjective and includes sensory, cognitive and affective components. As defined by the International Association for the Study of Pain (IASP), pain is “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (IASP, 1994). There is much interest in developing valid and reliable techniques for the objective assessment of pain in animals.

Methods of pain assessment in animals include measures of general body functioning (e.g. feed intake), physiological response measures (e.g. change in cortisol levels), as well as changes in behaviour (Weary et al., 2006). Pain-related behaviours might be observed for days to weeks after a painful procedure due to tissue damage, inflammation and repair. Behavioural responses will vary among species, but may include altered posture (e.g. time spent lying down or standing), changes in specific movements (e.g. trembling, tail or ear-flicking, kicking, slower locomotion), reluctance to feed and lethargy. Some animals display noticeable pain-related behaviours (e.g. vocalizations by pigs during castration: White et al., 1995; kicking and abnormal lying positions in calves due to castration: Molony et al., 1995). Other animals do not show such responses; stoicism may be a survival mechanism for prey species that are in danger of alerting predators. Pain-specific behaviours may occur during a procedure. For example, at the time of hot-iron branding cattle show escape-avoidance reactions (Lay et al., 1992) and behaviours such as tail flicking, kicking, and falling (Schwartzkopf-Genswein et al., 1997). Particularly relevant to the current study is research on responses following abdominal surgery. Research on rats (Roughan and Flecknell, 2001) and cats (Waran et al., 2007) have identified specific behaviours that emerge in the hours after surgery, including back arching, writhing, twitching and crouching. These behaviours were reduced or eliminated with effective doses of analgesics.

1.2. Aim

The aim of the current study was to describe the specific behavioural responses that occur in juvenile Steller sea lions following abdominal surgery for the implantation of telemetry devices, as a first step in understanding pain responses in this marine mammal. These sea lions were captured and underwent abdominal surgery as a part of a separate larger project. However, the current permitting guidelines for this endangered species did not allow for specific treatment groups (e.g. with and without analgesics). Under these conditions, we tested the primary hypothesis that behaviour measured pre-surgery would differ from post-surgery, and these differences would return to baseline by the late post-surgery period.

2. Methods

2.1. Study design and animals

This study was conducted at a quarantine facility at the Alaska SeaLife Center (ASLC) in Seward, AK, USA, as a part of the larger Transient Juvenile Steller Sea Lion Project (Mellish et al., 2006). The facility consists of four adjoining pools each enclosed by a metal surface haul-out area. A chain link fence surrounds each pool such that animals can be housed individually or share access to multiple pools via sliding gates, as research and husbandry protocols require.

Free-ranging juvenile Steller sea lions, between 16 and 23 months of age, included in this study were captured in Prince William Sound, AK, USA, as described by Mellish et al. (2006). Animals were from two separate capture groups; Group 1 was captured in August 2007 and contained five males and Group 2 was captured in February 2008 and contained three males and one female. Animals were transported to the ASLC for up to 3 months of research, including abdominal surgery for implantation of life history transmitters (LHX) tags, and were housed together to the maximum extent possible. The sea lions were uniquely identified with symbols shaved in their fur on their dorsal side to facilitate identification prior to permanent marking.

All animals were implanted with two LHX tags 5 weeks after capture. Sea lions were alternately assigned to hot-iron branding immediately following LHX tag surgery (five sea lions) or to the LHX tag surgery alone (four sea lions). All LHX implantation and hot-iron branding events were performed under the Transient Juvenile Steller Sea Lion Project (Mellish et al., 2006), with no directed or additional handling required to achieve our monitoring goal for this study. All animals were released after 9 weeks in captivity. Research was approved under Institutional Animal Care and Use Committee protocols AUP07-009 (ASLC), A07-0342 (UBC), 08-26 (UAF) and NMFS permit #881-1890-01.

2.2. Study procedures

The LHX tags used in this study are archival, satellite-linked telemetry devices specifically designed for the life-long monitoring of pinnipeds and are described in detail in Horning and Hill (2005). LHX tags are cylindrical (122 mm

in length, 42 mm diameter) with a mass of 115 g. The experimental protocol of the LHX tag study required two transmitters per study animal (Horning and Hill, 2005; Horning et al., 2008).

LHX tags were implanted by ventral midline laparotomy into the ventrocaudal abdominal cavity under isoflurane inhalant gas anaesthesia (as described in Horning et al., 2008), with an average duration of anaesthesia 137.3 ± 6.2 min (mean \pm S.E.M.). All animals received the systemic non-steroidal anti-inflammatory analgesic flunixin meglumine (Banamine[®]) administered at 1 mg/kg per total body mass intramuscularly into the hip region immediately prior to extubation (onset within 2 h, duration 12–24 h). No complications due to surgery were noted and all animals recovered from anaesthesia without incident. At the attending veterinarians direction, the analgesia protocol was modified midway through the study; such the four sea lions from Group 2 also received a line block local anaesthetic combination of 2 mL lidocaine and 1 mL bupivacaine (lidocaine – onset 3–5 min, duration 60–120 min; bupivacaine – onset 6–20 min, duration 200 min) via subcutaneous injection immediately prior to and alongside the first incision. This provided the unexpected opportunity to compare animals with and without line block analgesia, with the researcher blind to the line block treatment.

Hot-iron brand marks consisted of a combination of four numerals (each 10.2 cm high and 5.1 cm wide). Each numeral was applied to the left shoulder/flank for 2–4 s each after the completion of surgery and while still under gas anaesthesia, as described by Mellish et al. (2007b).

2.3. Behavioural observations

All behaviours were monitored for 9 days: 3 days before surgery, days 0–2 immediately following surgery, and again in days 10–12 after surgery (nominally pre-, post- and late post-). With the exception of the day of surgery,

focal sampling occurred on all animals six times a day in 10-min periods, twice during each of the following periods of the day: 09:00–11:00 h, 13:00–15:00 h and 17:00–19:00 h. On the day of surgery, focal animals were observed for 1 h after surgery (which took place within 1.5 h after being extubated from anaesthesia, while being held in a dry holding area between pools). Steller sea lions typically return to activities, such as locomotion, within 1 h after isoflurane gas anaesthesia (Heath et al., 1997). After this first hour of observation, 10-min observations resumed. All animals were observed for the same amount of time and observations were equal across the 3-day parts, with the exception of day 10, which has missing observations from three animals. Behaviours were recorded live by one observer. This observer had extensive experience scoring these behaviours in sea lions. The observer was sheltered from the sea lions' view, either behind a plastic blind or via a one-way window depending upon the location of the focal animals.

The behaviours listed in Table 1, as well as lying and sitting position (dorsal, ventral or on their side) and proximity to others, were recorded using point-in-time sampling (one sample every 1 min for 10 min). Mutually exclusive behaviours included locomotion (which includes both on land and in the water), sit upright, lie down, stand, groom, and float. Mean proportion of time on ventral side was measured during the time sea lions spent sitting upright and lying down while on land. Mean proportion of time back arching was calculated from sea lions that displayed back arch behaviour on land during periods of sitting upright and lying down. Mean proportion of time spent in the pool was calculated from activities that occur in the water (i.e. locomotion, floating and foraging).

To determine sample size required for behaviours to provide information on the effects of LHX implant surgery, power calculations were computed using preliminary results from August 2007 data (using Piface version 1.63 software). For the 11 behaviours listed in Table 1, the

Table 1
Descriptions of behavioural activities recorded before and after LHX implant surgery.

Behaviour	Description
Land and water behaviours	
Alert	Attentive with both eyes open
Locomotion	Moving on the ground or in water (i.e. swimming by actively propelling itself through the water by means of movement of the body)
Land only behaviours	
Sit upright	Weight placed on back flippers and/or lower body, upper body lifted off the ground, front flippers touching ground but not bearing the majority of the animal's weight
Lie down	Sea lion is in a flat or horizontal position to the ground, while on their ventral, dorsal, or lateral side, head may be lifted or on the ground
Stand	Weight is distributed among all four flippers, quadrupedally, that are positioned underneath the sea lion's body, belly lifted off the ground, head up
Back arch	Dorsal curvature of the spine (non-linear) in the lower thoracic and lumbar vertebrae region while lying down or sitting upright, belly lifted up off the ground
Grooming	
Scratch	Use of flipper to scrape at skin
Bite	Use of teeth to grip or hold an area of body, usually witnessed in a fast repetitive motion
Body rub	Moves part of body back and forth with friction and pressure on the ground, fence, wall, dry mat or on another sea lion
Head rub	Moves head back and forth with friction and pressure on another area of its own body
Water only behaviours	
Float	Suspended in the pool, either free-floating or with flippers hanging on to side of the pool

analysis determined that a sample size of between three and eight individuals would be required to accurately identify the behavioural effects of LHX implant surgery.

To establish if the chosen sampling method accurately represented the proportion of time spent in each of the behaviours listed in Table 1, a validation study was conducted on three animals for a full day before and after the procedure. The estimates generated using our sampling method (six 10-min sampling periods a day) were compared to the total daily proportion of time spent in each behaviour using regression. Only behaviours with a regression coefficient of 0.80 or higher were included in the study (pooled $R^2 = 0.94$, range 0.80–0.99). On this basis, the behaviours sit upright, groom and float were excluded from the analysis.

2.4. Statistical analysis

Days were calculated using 24-h periods, with day 0 starting immediately following extubation from anaesthesia. The proportion of time spent displaying each behaviour was averaged across both the pre-surgery, post-surgery and the late post-surgery days to generate one measure per animal per period. Proportional data were outside the range of 0.3–0.7. Therefore, to condense the distribution and to allow for use in the statistical analyses, all data were arcsine square root transformed ($Y = \arcsin \sqrt{p}$). Mixed model analysis (SAS v9.1) was conducted to test the effects of LHX implant surgery day on the various behavioural activities. The analysis included animal as a random effect and tested for linear effects of day. The model included a within-subject factor (day: pre-, post- and late post-surgery) and two between-subjects factors (branding: yes or no; Group: 1 or 2). The residuals from the models were tested against the basic assumptions of normality and variance homogeneity. Two specified contrasts were run to compare pre- vs. post-surgery and pre- vs. late post-surgery periods. In all cases, differences were considered to be significant at $P \leq 0.05$.

3. Results

Changes in sea lion behaviour were noted for six parameters (stand, back arch, time spent on ventral side,

locomotion, time spent alert, and lying time; Table 2). In particular, there was an effect of day for two behaviours that were rarely observed prior to surgery: standing and back arching ($F_{2,12} = 48.18$, $P < 0.001$ and $F_{2,12} = 128.98$, $P < 0.001$, respectively). Time spent standing and with the back arched was higher post-surgery than pre-surgery ($F_{1,12} = 85.97$, $P < 0.001$ and $F_{1,12} = 246.92$, $P < 0.001$, respectively; Fig. 1a). Standing and back arch peaked post-surgery, but still occurred in the late post-surgery period ($F_{1,12} = 29.71$, $P = 0.001$ and $F_{1,12} = 68.05$, $P < 0.001$, respectively; Fig. 2).

There was a significant effect of day on the time sea lions spent with pressure on their ventral side ($F_{2,12} = 127.93$, $P < 0.001$; Fig. 1b). During periods of lying and sitting, sea lions spent less time with pressure on their ventral side post-surgery ($F_{1,12} = 208.44$, $P < 0.001$); this decrease was still witnessed in the late post-surgery period ($F_{1,12} = 172.03$, $P < 0.001$). Sea lions instead switched to lying and sitting on their left and right sides. There was an effect of day for locomotion behaviour ($F_{1,12} = 3.82$, $P = 0.05$; Fig. 1c). When compared with the pre-surgery period, sea lions spent less time post-surgery in locomotion ($F_{1,12} = 4.71$, $P = 0.05$) and this response returned to baseline by the late post-surgery period.

Sea lions tended to spend less time alert and more time lying down after LHX surgery ($F_{2,12} = 3.11$, $P = 0.08$ and $F_{2,12} = 2.99$, $P = 0.09$, respectively). Specifically, sea lions spent less time alert in the post-surgery period compared with pre-surgery ($F_{1,12} = 7.05$, $P = 0.02$), with this response returning to baseline by the late post-surgery period. There was a tendency for sea lions to spend more time lying down post-surgery when compared with pre-surgery ($F_{1,12} = 3.87$, $P = 0.07$), with this increase from pre-surgery more evident in the late post-surgery period ($F_{1,12} = 6.36$, $P = 0.03$). There was no effect of day on time spent in the water.

For all behaviours there was no effect of branding additional to that of the surgery.

When comparing sea lions from Group 1 with Group 2, there was an interaction between group and day for standing behaviour ($F_{2,12} = 3.97$, $P = 0.047$). Sea lions from the Group 2 spent less time standing in the post- and late post-surgery periods than the sea lions from the Group 1 (Fig. 2). No other group interactions were significant.

Table 2

Least square means and S.E.M. for the proportion of time sea lions ($n = 9$) spent displaying behaviours 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 $P \leq 0.05$.

	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

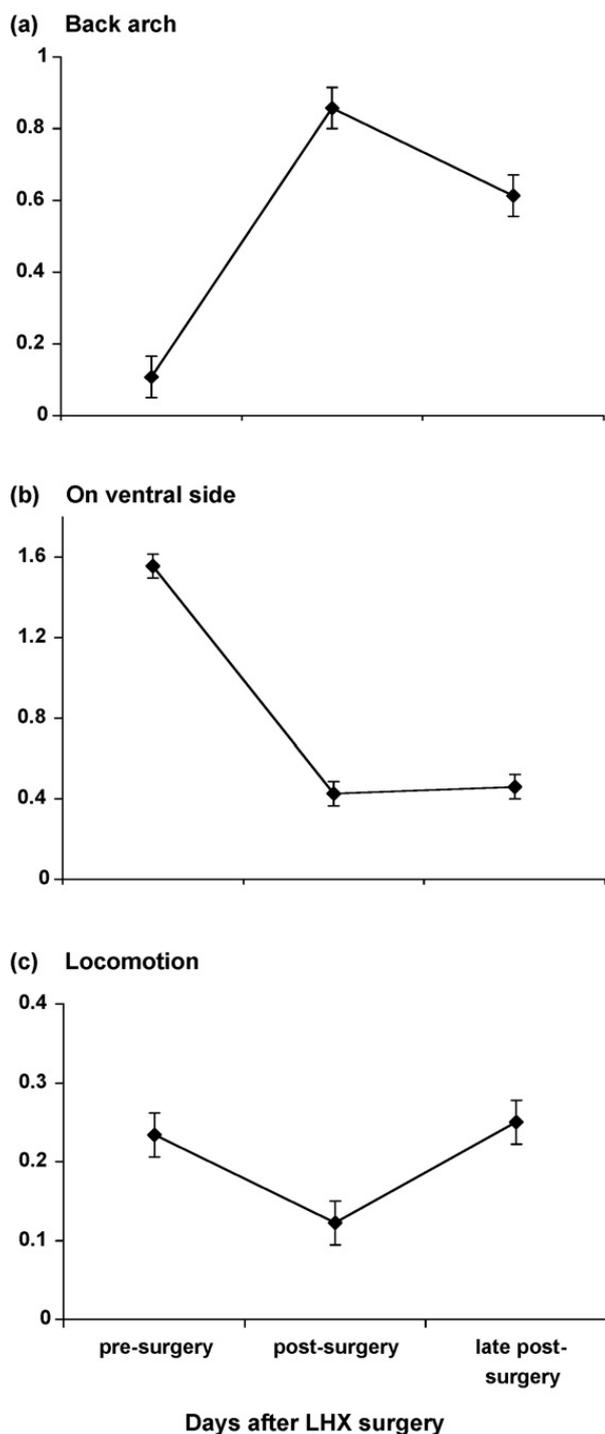


Fig. 1. Least square means (\pm S.E.M.) for the proportion of time sea lions spent (a) displaying back arch behaviour while sitting upright and lying down while on land, (b) with pressure on the ventral side during periods of lying down or sitting upright while on land, and (c) in locomotion on both land and in the water. Means and S.E.M. are the arcsine square root transformed values. The x-axis represents time, presented as pre-surgery (average of 3 days before surgery), post-surgery (average of the 3 days immediately following surgery), and late post-surgery (average of days 10–12 following surgery). Day effects on back arch, time on ventral side, and locomotion were significant at $P < 0.001$, $P < 0.001$ and $P = 0.05$, respectively.

4. General discussion

This study provides the first description and analysis of post-operative behavioural responses to surgery in a

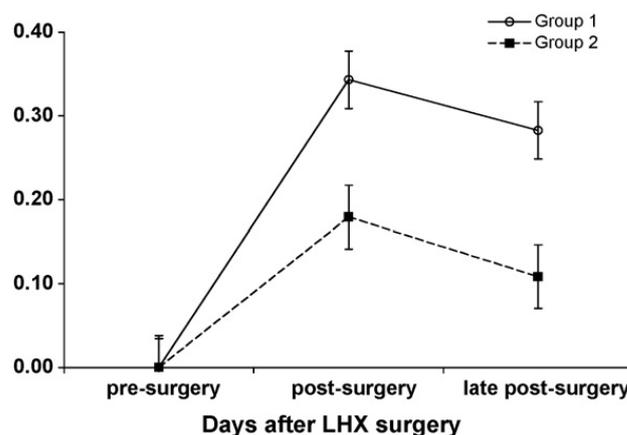


Fig. 2. Least square means (\pm S.E.M.) for the proportion of time sea lions spent standing on land before and after LHX surgery. Means and S.E.M. are the arcsine square root transformed values. Open circle represent animals from Group 1 ($n = 5$) and closed squares represent animals from Group 2 ($n = 4$). The overall effect of day on standing behaviour was significant at $P < 0.001$. The interaction between line block and day on standing behaviour was significant at $P = 0.047$.

marine mammal. 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 never or rarely observed before surgery. Time spent with pressure on the ventral side where the incision site was located decreased following surgery; sea lions instead switched to lying and sitting on their sides. Sea lions may use these postures to reduce stimulating the area of the injury. The tissue and nerve damage, as well as local inflammation, may have increased the activity of peripheral nociceptors and peripheral or central sensitization (Vinuela-Fernandez et al., 2007). An increased sensitivity to pain, or hyperalgesia, can occur due to the local release of inflammatory mediators and cytokines and is a common feature of inflammatory pain (Coderre and Melzack, 1987). Primary hyperalgesia develops at the site of the injury while secondary hyperalgesia develops in the surrounding uninjured tissue (Meyer et al., 2006). Hyperalgesic effects in cattle with mastitis persist between 4 and 20 days depending on the severity of the mastitis (Fitzpatrick et al., 1999) and for at least 5 weeks in mice with amputated tail tips (Zhuo, 1998). In the current study, back arch and standing behaviours were both reduced, but not completely eliminated by the late post-surgery period, indicating that animals may be still recovering from surgery and hyperalgesic effects may be present.

Prolonged back arch and standing responses may not only result from the surgical incision, but in response to the movements of the free-floating LHX tags within the abdominal cavity. However, rats and cats who have undergone abdominal surgery, but with no internal placement of a tracking device, display similar back arching (Roughan and Flecknell, 2001, 2004) and 'half-tucked-up' and crouching behaviours (Waran et al., 2007). Control surgery, comparing animals that undergo surgery with LHX implants vs. animals that undergo surgery but do not receive an LHX implant (i.e. incision only), would help

identify the potential cause of these post-operative differences. These treatments were not possible in this study, given the current permitting restrictions (NMFS permit #881-1890-01).

Pain can also affect locomotion activity (Flecknell and Liles, 1991) and lying behaviour (Hemsworth et al., 2009). In the current study we found a significant reduction in locomotion both on land and in the water in the post-surgery period, with levels returning to baseline by the late post-surgery period. Sea lions also tended to spend more time lying down in the post- and late post-surgery periods compared to the pre-surgery period. The location of the abdominal wound may play a key role in potential restriction of movements such as lifting the flippers and rotating the body. Reduced locomotion and increased lying time may also be explained by the presence of inflammation and the inflammatory pain associated with the local release of inflammatory mediators and cytokines, or may simply be due to the animals adjusting to their activity levels after being in captivity for more than a month. An incision of 9–12 cm in length is required to insert the LHX tag into the abdomen. Smaller incision sites may be possible for applications of smaller telemetry implants. Alternatively, a change in the location of the incision site from the ventrocaudal region to the right or left side flank may alter or reduce some of these responses.

Within the limited sample size and the constraints of the behavioural comparisons performed, we found no evidence of an effect of branding additional to that of the surgery. Hot-iron branding occurred as a part of the Transient Juvenile Project as described in Section 2 and was not an intentional part of the design of our surgery assessment. We suggest that future studies on the effects of hot-iron branding should use a more sensitive within-subject design and should not include animals recovering from surgery.

Behavioural responses differed between Group 1 and Group 2. Sea lions in the Group 2 spent less time standing after surgery. This difference may have been due to the lidocaine–bupivacaine line block administered to this group, or to any effects of group composition or time of year. Well-controlled studies on the effects of different analgesic protocols are still required.

The behavioural differences described above cannot be definitively associated with pain, and the study was not designed to assess how much pain the animals were experiencing. Instead, the likely association between observed behavioural changes and pain should be considered as hypotheses to be tested by specific further investigations. For example, comparing changes in time budgets before and after surgery is a useful first step, but stronger conclusions will require comparison of untreated control groups (surgery and no analgesia), and analgesia and anaesthetic control groups (with no surgery; Flecknell and Roughan, 2004). Some approaches that are ideal scientifically may not be suitable for use in an endangered species like the Steller sea lion; for example, the inclusion of untreated controls group is likely not possible from a permitting standpoint. Adjustments to analgesic protocols using the current procedures as a positive control, is a preferred option. For example, some research suggests that

combined pre- and post-operative analgesic treatment is more effective than pre-operative treatment alone (Dobromylskyj et al., 2001; Waran et al., 2007). The aim in pre-emptive analgesia administration is to reduce the firing of nociceptors and thus the hyper-analgesia induced by over-sensitization caused by damaged tissues (Dobromylskyj et al., 2001). Increased dosage, longevity of regional and systemic analgesics, or pre-operative analgesia may allow a reduction in post-operative pain as inferred from the observed changes in behaviour. Further research with alternative drugs and dosages is needed to accurately define a maximally effective and safe analgesia treatment protocol for this species.

5. Conclusion

In the days after abdominal surgery, Steller sea lions spent more time with their back arched and standing, and spent less time lying on the ventral side and in locomotion. These behavioural responses suggest that the animals may be attempting to minimize post-operative pain by avoiding stimulation of the incision site. Moreover, these results suggest that these responses should be useful in monitoring pain following similar surgeries in marine mammals. Behavioural responses to surgeries suggest that additional pain management strategies (i.e. alternative analgesia, increased dosage, or pre-operative administration of analgesia) should be investigated.

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