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Monitoring Glucocorticoid Response to Rehabilitation and Research Procedures in California and Steller Sea Lions

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ABSTRACT We used serum and fecal corticosteroid analysis to study the physiological response to a range of invasive and non-invasive procedures in sea lions. Four experimental groups of California sea lions (*Zalophus californianus*; Group A: restraint only [$n = 9$], Group B: gas anesthesia without surgery [$n = 10$], Group C: minimally invasive surgery [$n = 10$], and Group D: invasive surgery [$n = 5$]) were monitored for adrenal response. Feces were collected opportunistically from 72 hr before 72 hr post procedure for corticosterone analysis. All experimental groups showed substantial individual variation and no significant change in corticosterone levels after the procedures. Additional fecal and serum corticoid samples were collected from six free-ranging Steller sea lions (*Eumetopias jubatus*) in temporary captivity undergoing abdominal implantation of long-term telemetry devices. Only one sea lion exhibited a significant fecal corticosterone increase in response to the surgery. Capture and restraint appear to elicit a greater glucocorticoid response than invasive or non-invasive procedures. *J. Exp. Zool. 309A:73–82, 2008.*

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Rehabilitation of marine mammals and applied research techniques require direct handling of individuals and are a potential source for stress (St. Aubin and Dierauf, 2001; Petrauskas et al., '06). Most marine mammals that enter rehabilitation undergo procedures ranging from non-invasive (e.g. fecal collection), minimally invasive (e.g. radiographs) to invasive procedures involving surgery and implantation of telemetry devices (Dover and Van Bonn, 2001; Haulena and Heath, 2001; Larson et al., 2002). Short-term effects of these procedures can be monitored through clinical indicators and possible long-term effects via post-release survivorship using tagging and telemetry (Williams and Siniff, '83; Mulcahy and Esler, '99; Dover and Van Bonn, 2001; Hernandez-Divers et al., 2001; Larson et al., 2002; Horning and Hill, 2005).

Stress physiology is the study of the body's response to alterations in homeostasis (Sapolsky, '92). A stressor can be physical, psychological, acute, or chronic, but all mammalian responses have a common pathway. Corticotropin-releasing hormone is released from the hypothalamus in

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response to a perceived stressor. Corticotropin-releasing hormone activates the anterior pituitary gland to release adrenocorticotropic hormone (ACTH), which in turn stimulates the adrenal cortex to secrete glucocorticoids. Glucocorticoids and catecholamines (epinephrine and norepinephrine) together mediate most of the changes that form the stress response. Glucocorticoids are metabolized by the liver (Dehnard et al., 2001). Major glucocorticoids in mammals are cortisol and corticosterone. After metabolism, cortisol (and its metabolites) returns to the blood stream, whereas corticosterone enters the bile and is excreted with feces (Möstl and Palme, 2002). Cortisol and corticosterone are the two hormones typically used to evaluate stress in marine mammals (St. Aubin and Dierhauf, 2001).

Serum cortisol has been the preferred media for stress evaluation in animals, but serum cortisol only offers a ''snapshot'' of the hormone level and may not truly monitor the ''stressed'' state of the animal. For instance, many species exhibit a diurnal rhythm in their glucocorticoid levels (Oki and Atkinson, 2004); the timing of sampling can become an issue when comparing glucocorticoid concentrations from different groups of animals. Chronic stress in sheep has been shown to alter or abolish those diurnal rhythms, and therefore frequent blood sampling is required for accurate interpretation (Przkop et al., '85). Fecal corticosterone analysis is an alternative, non-invasive method to monitor glucocorticoids in organisms. Fecal samples are easily collected and provide an integrated response that represents longitudinal data over time, as opposed to serum cortisol levels that can change quickly from sample to sample (Möstl and Palme, 2002). Fecal corticosterone assays have been validated to monitor adrenal activity in many animals: large terrestrial mammals (Czekala et al., '94; de Villiers et al., '95; Monfort et al., '96; Terio et al., '99; Wasser et al., 2000; Turner et al., 2002); ruminants (Palme and Möstl, '96; Wasser et al., 2000; Millspaugh et al., 2002; Morrow et al., 2002; Huber et al., 2003); rodents (Harper and Austad, 2000); birds (Ludders et al., 2001; Wasser et al., 2000); hares (Teskey-Gerstl et al., 2000), and marine mammals (Wasser et al., 2000; Mashburn and Atkinson, 2004).

Monitoring glucocorticoid concentrations in marine mammals can give insight into the degree of stress response associated with veterinary and research procedures, which can allow management protocols to be modified to effectively reduce anxiety. Research conducted on otariids (sea lions and fur seals) to date indicates a lack of cortisol response to isoflurane anesthesia in captive, adult Steller sea lions (*Eumetopias jubatus*; Mashburn and Atkinson, 2004) and a predictable glucocorticoid response to various acute stressors and surgical procedures in a single rehabilitated Steller sea lion pup (Petrauskas et al., 2006). Most studies of the effects of telemetry device implantation in wild and captive animals only investigate the immediate behavioral and physical responses to the procedures. Such studies have been performed in European badgers (*Meles meles*; Ågren et al., 2000), North American river otters (*Lontra canadensis*; Hernandez-Divers et al., 2001), and sea otters (*Enhydra lutris*; Williams and Siniff, '83). In one instance, post-release mortality was studied in harlequin ducks (*Histrionicus histrionicus*) outfitted with abdominal radio transmitters (Mulcahy and Esler, '99).

The objectives to this study were (1) to compare stress hormone concentrations associated with different rehabilitation procedures in California sea lions (*Zalophus californianus*) through the use of fecal corticosterone analysis, (2) to determine stress hormone concentration levels over the course of temporary captivity of wild juvenile Steller sea lions (*E. jubatus*), using serum cortisol measurements, and (3) to assess the stress response to intraperitoneal telemetry tag implantation in California and Steller sea lions using fecal corticosterone analysis.

MATERIALS AND METHODS

California sea lions

Four experimental treatment categories (Table 1) were established from existing, routine rehabilitation procedures used in California sea lions at The Marine Mammal Center (TMMC), Sausalito, CA. Rehabilitation was required owing to trauma, abscesses, *Leptospirosis* sp. infection, pneumonia, domoic acid exposure (Scholin et al., 2000), and malnutrition (Greig et al., 2005). Animals were housed in primary outdoor enclosures with access to water. Access to conspecifics was whenever possible. The sea lions were fed by tossing freshly thawed fish three times a day into each pool. Maintenance, feeding, sanitation, and management were accomplished with minimal human contact. Sea lions were from two stages of rehabilitation: ''admit'' and ''pre-release.'' Admit animals were new to TMMC, exhibited clinical signs of illness, and were considered in

TABLE 1. Total number of each sex and age class of California sea lions used to evaluate the effects of physical restraint (Group A), inhalant gas anesthesia for non-surgical procedures (Group B), minimally invasive surgery (Group C), and invasive surgery (Group D) on fecal corticosterone concentration

	Male				Female		
Group ID	Yearling	Juvenile	Subadult	Adult	Yearling	Juvenile	Adult
A	1	2	1	1	1	2	1
B	5			1	2	1	1
C	3	3			4		
D			3	1			1

compromised health by the veterinary staff. Pre-release sea lions were medication free, had adequate appetite and were considered releasable by the attending veterinarian.

Experimental groups

Group A, restraint only ($n = 9$): animals in this group were physically restrained for a blood sample for regular health assessments. Herding boards, a towel to cover the animal's eyes, and a person straddling the animal allowed a technician to draw blood from the caudal plexus vein. Light pressure was applied to the shoulders and head of the animal.

Group B, gas anesthesia without surgery ($n = 10$): animals in this group were immobilized through inhalant gas anesthesia for non-surgical purposes after intra-muscular chemical sedation with medetomidine (0.04 mg/kg) and telazol (1.0 mg/kg; Haulena and Gulland, 2001). Herding boards were used to protect handlers during initial injection. Gas anesthesia was applied for 5–40 min and sedation was subsequently reversed with an intra-muscular injection of atipamezole (0.2 mg/kg; Haulena and Gulland, 2001). Typical procedures during the anesthesia event included radiographs, wound examination, or ophthalmic examination.

Group C, minimally invasive surgery ($n = 10$): animals in this group were chemically sedated and reversed as described above for Group B. Isoflurane anesthesia duration was from 15 to 150 min. Procedures included lancing of abscesses with drain placements ($n = 3$) and third eyelid suturing to treat corneal ulcerations ($n = 7$).

Group D, invasive surgery ($n = 5$): animals in this group were chemically sedated and reversed as described as above. Isoflurane anesthesia duration ranged from 80 to 105 min. Treatment procedures included the intraperitoneal implantation of Life History Transmitter (LHX) tags ($n = 4$; for a description of LHX tags, see Horning and Hill, 2005) and laparoscopy ($n = 1$). LHX recipients were healthy at the time of surgery. The sterilized transmitters were inserted through a ventral incision between the caudal sternum manubrium and the pubic bones into the abdominal cavity. Standard aseptic surgical techniques were used.

Stress levels associated with the different treatments were assessed through fecal corticosterone analysis. Fecal samples for all groups were collected opportunistically up to 72 hr before and 72 hr post procedure.

All procedures at TMMC were carried out under NMFS permit 1034-1685-01 and TMMC Animal Use Protocols 02/4 and 05/3.

Steller sea lions

Six juvenile Steller sea lions (14–16 mo; 5 m, 1 f) were collected in Prince William Sound, Alaska, between September 2005 and February 2006 as part of a larger research program (see Mellish et al., 2006). Animals were held in a specialized outdoor quarantine facility at the Alaska SeaLife Center (ASLC), Seward, AK and kept under temporary captivity for 10–12 weeks before release. Animals were maintained in primary outdoor enclosures with access to pools and conspecifics whenever possible. Freshly thawed fish was offered several times a day via remote methods to minimize associating food with husbandry personnel. Animals were conditioned to move through remotely operated gates, holding areas, and cages by ''context-specific conditioning.'' This training technique allowed for the management, feeding, sanitation, and maintenance to be accomplished with minimal human contact (Mellish et al., 2006).

Fecal samples were collected whenever possible without disturbing the animals on an opportunistic basis over the entire captivity period. Serum samples were collected from sea lions on the day of capture, 7 d before implantation, day of implantation, 7, 14, 21, 28, 35, and 42 d post implantation. All serum samples, except the capture collection samples, were taken within 10–15 min of the animal being brought into the squeeze cage. The time between capture in the wild to blood sampling was greater than 2 hr. All animals were monitored for 4–6 weeks after LHX tag implantation. LHX implantation procedure was similar as described above for California sea lions of Group D, without administration of chemical sedation. For all research procedures, from serum sample collection to LHX implantation, animals were physically restrained using a squeeze cage for direct inhalant gas induction using a large face mask.

All procedures at ASLC were carried out under NMFS permit 881-1668 and ASLC Animal Use Protocols 02-015 and 03-007.

Fecal sample extraction and preparation

To extract corticosterone from fecal material, all samples were fully mixed, aliquoted (~5 g), loaded onto a rotary evaporator (Speed-Vac Plus, SC110A; Savant Instruments, Holbrook, NY), and dried without heat. Dried fecal samples were crushed into powder and 0.025 g ($\pm$0.001) was weighed and extracted as previously described by Monfort et al. ('96). Methanol (MeOH) extractant (100 μL) was aliquoted into polypropylene tubes, dried under forced air and reconstituted in 900 μL buffer for a final 1:10 dilution. Sample dilutions were stored frozen at −20°C until radioimmunoassay (RIA). For a random set of samples, another 100 μL of MeOH extractant was used to calculate extraction efficiency. Calculation was determined by ''spiking'' random samples with 10μL of tritiated corticosterone at the crushed fecal powder stage. After extraction, 100μL of MeOH extractant was added to scintillation vials along with 3 mL of Ultima Gold scintillation cocktail (PerkinElmer, Boston, MA), and radioactivity was counted for 2 min/vial using a Beckman LS6500 liquid scintillation counter with a quench curve correction. Radioactivity in fecal extracts was converted to percent of the average total radioactivity added, and assay results were corrected accordingly.

RIA validation—fecal corticosterone

Each RIA was validated with standard methods including high-pressure liquid chromatography (HPLC). A fecal pool was created for both male and female sea lions to validate the corticosterone assay. Serial dilutions (neat to 1:1,024) of both sex pools were created to determine displacement parallel to the standard curve.

For HPLC (Varian ProStar 210/215, Varian, Walnut Creek, CA), both male and female fecal pools were spiked with tritiated cortisol and corticosterone as reference tracers. The samples were eluted through a reverse phase C-18 column using an organic/aqueous solvent gradient of 20–80% MeOH/H_2O to 100% MeOH over 80 min with a flow rate of 1 mL/min, with each milliliter collected as a separate fraction. After HPLC, 100 uL of each fraction was placed in a scintillation counter to determine the fraction in which the cortisol and corticosterone were eluted. The remaining portions of each fraction were divided into equal aliquots of 450 μL each, dried down, then brought up in the appropriate amount of buffer. Each aliquot was then run in both cortisol and corticosterone RIAs to determine immunoreactivity present in each fraction.

Fecal corticosterone RIA

A double antibody RIA kit (MP Biomedicals, Costa Mesa, CA) was validated for use in California sea lions and previously validated for use in Steller sea lions by Mashburn and Atkinson (2004) using ^{125}I for fecal corticosterone analysis. Radioactivity was determined using a gamma counter (Gamma C12, Diagnostic Products, Los Angeles, CA). Standard curves of RIAs were log–logit transformed to read the hormone concentrations off the standard curve (Rodbard, '74). Values from the RIA were corrected for dilution, extraction efficiency, weight of fecal material extracted, and expressed as ng/g dry weight. RIAs were performed according to manufacturer's instructions with the exception that all volumes were halved and an additional standard was added to the curve (i.e. one-half the lowest standard) to increase sensitivity. Manufacturer cross-reactivity with other steroids was as follows: desoxycorticosterone (0.34%), testosterone (0.10%), cortisol (0.05%), aldosterone (0.02%), and $<0.01\%$ for all other steroids tested. Samples were randomly assayed and inter-assay coefficient of variation for the assay control was 18.3% ($n = 22$ for all samples assayed) for California sea lion samples and 10.4%

($n = 9$ for all samples assayed) for Steller sea lion samples. Intra-assay coefficients of variation were $<5\%$ for all assays performed. Assay sensitivity for California and Steller sea lion samples was 13.7 and 14.4 ng/tube, respectively.

Serum cortisol RIA

All sera were collected via the caudal plexus or hind flipper vein into serum separator vacutainers in conjunction with routine blood collection (Mellish et al., 2006).

A solid phase RIA kit (Diagnostic Products, Los Angeles, CA), previously validated for use in unextracted, undiluted Steller sea lion serum (Mashburn and Atkinson, 2004) was used for cortisol analysis. Radioactivity was determined using a gamma counter (Gamma C12, Diagnostic Products, Los Angeles, CA). Standard curves of RIAs were log–logit transformed to read the hormone concentrations off the standard curve (Rodbard, '74). The RIAs were performed as per manufacturer instructions except that all volumes were halved. Manufacturer cross-reactivity data are as follows: prednisolone (76.0%), 11-deoxycortisol (11.4%), prednisone (2.3%), cortisone (0.98%), corticosterone (0.94%), tetrahydrocortisol (0.34%), 11-deoxycorticosterone (0.26%), aldosterone (0.03%), progesterone and pregnenolone (0.02%), flumethasone (0.017%), and 0.01% or below for all other steroids tested. Samples were randomly assayed and inter-assay coefficients of variation for the three separate assay controls were 4.3, 9.9, and 6.4% ($n = 4$ for all samples assayed). Intra-assay coefficients of variation were $<5\%$ and assay sensitivity was 5.75 ng/tube.

Baseline serum cortisol concentration was calculated using samples that were not affected by LHX surgery; therefore, samples collected within 21 d of those procedures were excluded from the calculations.

Data analysis

Time 0 or T_0 indicates the time of procedure for all experimental groups: CSL Groups A–D and SSL LHX implant groups. Results from analysis of fecal samples for California sea lion groups A–D were analyzed using the Wilcoxon Signed-Rank Test; n.s., abbreviation for not significant. Results from the admit versus pre-release CSL fecal corticosterone concentrations at T_0 were compared using the Mann–Whitney Rank Sum Test. Data from serum samples for all Steller sea lions were analyzed with a Wilcoxon Paired Sample Test. The null hypothesis of no change for all tests was rejected at $P < 0.05$. The statistical software used was SPSS 14.0 for analysis of data and StudySize 2.0 for power analysis.

RESULTS

RIA validation–fecal corticosterone

Serial dilutions (neat to 1:1,024) of both pools of male and female feces yielded displacement parallel to the standard curve. Recovery of added corticosterone (range 125–5,000 ng/mL) was 107.1% (SD = 17.8; CV = 16.7%) for females ($y = -5.24 + 1.05x$, $r^2 = 1.0$) and 100.6% (SD = 11.7; CV = 11.7%) for males ($y = 1.49 + 1.02x$, $r^2 = 1.0$).

HPLC—fecal corticosterone

Fecal aliquots run in a corticosterone RIA co-eluted in association with the corticosterone peak and represented 66.8 and 31.2% of the total mass eluted for females and males, respectively.

Fecal corticosterone

California sea lions of all experimental groups that were part of the ''admit'' group at the time of procedure had significantly higher pre-procedure fecal corticosterone concentrations than ''pre-release'' ($P = 0.002$; $T_{0.05(1),32}$; Fig. 1).

None of the four California sea lion experimental Groups A–D had significantly different fecal corticosterone concentrations pre-procedure compared with post procedure: Group A ($P = 0.173$, $W_{0.05(1),9}$, Power = 0.0084; n.s.); Group B

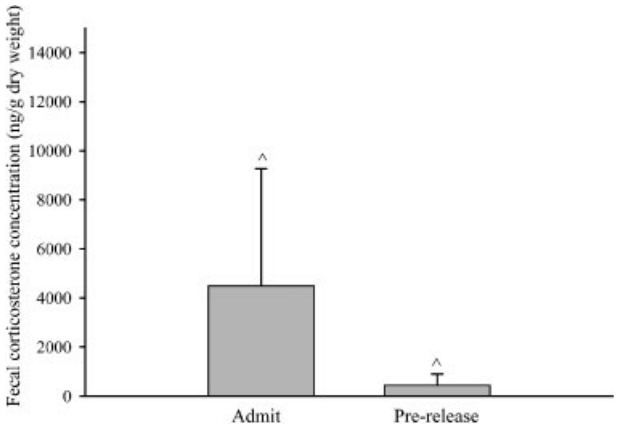

Fig. 1. Mean (±SE) fecal corticosterone concentration (ng/g dry weight) for all California sea lions in this study based on stage of rehabilitation at the time of the procedure. (∧) denotes a significant difference.

($P = 0.799$, $W_{0.05(1),10}$, Power = 0.7991; n.s.); Group C ($P = 0.203$, $W_{0.05(1),10}$, Power = 0.2022; n.s.); Group D ($P = 0.225$, $W_{0.05(1),5}$, Power = 0.2247; n.s.).

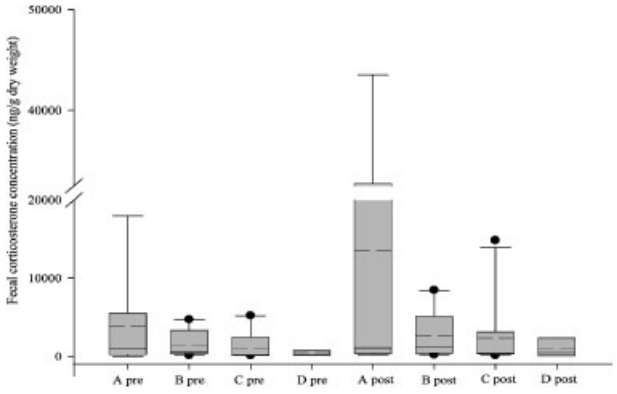

Fig. 2. Box plot (±SE) comparisons of California sea lion fecal corticosterone concentrations (ng/g dry weight) pre-treatment and post treatment for each experimental group (A, physical restraint; B, inhalant gas anesthesia for non-surgical purposes; C, minimally invasive surgery; D, invasive surgery). Solid line (-) within the box indicates median, dashed line (– –) within the box indicates mean, and solid circles indicate outliers.

Descriptively, changes in mean pre- versus post-treatment corticosterone values for groups, were greatest in Group A followed by Groups C, D, and B (Fig. 2).

Of the six animals, only one juvenile Steller sea lion descriptively exhibited a clear glucocorticoid response to LHX implant surgery (Fig. 3). A glucocorticoid response should be measured in the 24–48 hr post-treatment fecal samples (Mashburn and Atkinson, 2004). However, not all feces produced by the experimental animals could be collected, therefore statistical tests were not performed on the small sample sizes.

Serum cortisol

Baseline serum cortisol concentration of the Steller sea lions in this experimental group was 74.41 ± 21.60 ng/mL (Fig. 4).

Serum cortisol concentrations from Steller sea lions were significantly higher at capture compared with baseline serum cortisol concentrations ($P = 0.028$; $W_{0.05(1),6,19}$; Fig. 4). Serum cortisol concentration from the day of the LHX surgery compared with 7 d post-LHX surgery was not significantly different ($P = 0.285$, $W_{0.05(1),6,3}$,

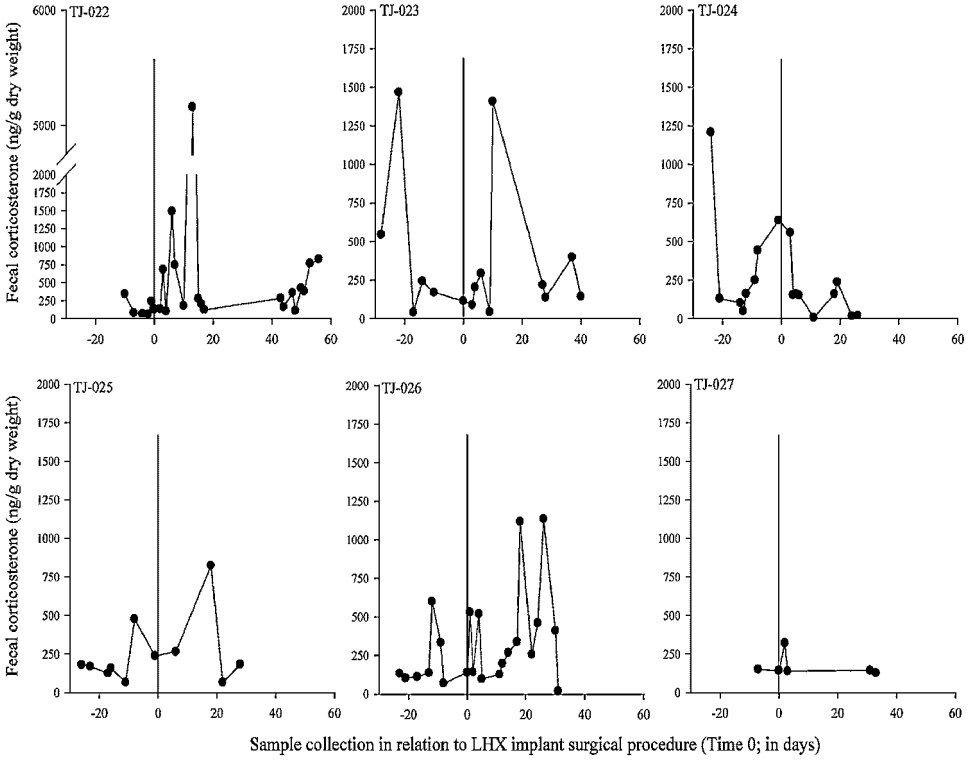

Fig. 3. Fecal corticosterone (ng/g dry weight) profiles over the entire course of temporary captivity for all six juvenile Steller sea lions. Sample collection time (in days) is in relation to LHX implant procedure as indicated by a vertical line at 0. *Note*: *y*-axis scaling is (TJ-022) 0–6,000 ng/g with a break in the *y*-axis between 2,000–4,000 ng/g and (TJ-023–TJ-024) 0–2,000 ng/g.

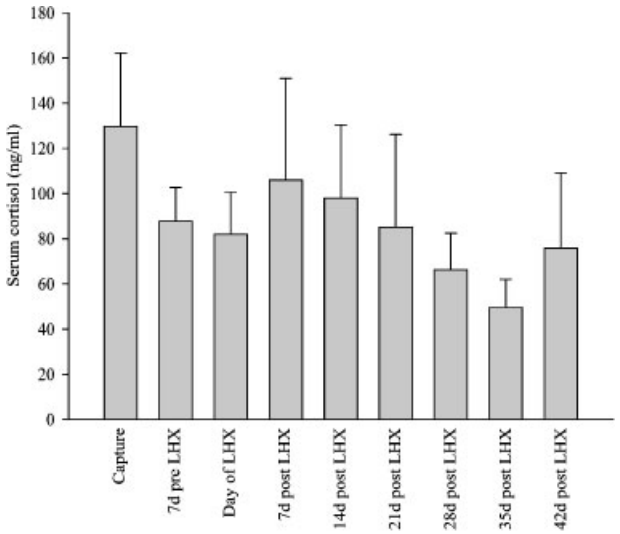

Fig. 4. Serum cortisol concentrations (ng/mL) for all sample collection times of the six juvenile Steller sea lions that underwent a major invasive surgery (LHX). Serum cortisol concentrations were significantly higher at capture compared with baseline serum cortisol concentration ($P = 0.004$; $W_{0.05(1),6,19}$; Fig. 4). *Note*: The baseline value was calculated using 7 d pre-LHX, 28 d post LHX, and 35 d post LHX.

Power = 0.1225) nor was a comparison between the mean of 7, 14, and 21 d post-LHX surgery and the mean of samples from 7 d pre-LHX, day of LHX surgery, and 28 d post-LHX surgery ($P = 0.345$, $W_{0.05(1),13,15}$, Power = 0.8303; Fig. 4).

DISCUSSION

Standard RIA validation techniques, including HPLC, supported the assumption that fecal corticosterone could be accurately measured in California sea lions. Further validation techniques, such as ACTH challenges, are required in order to biologically validate the use of fecal corticosterone RIAs. ACTH challenges allow for the quantification of maximum adrenal output and timing of adrenal output in animals. Previous ACTH challenges in marine mammals are limiting. Hunt et al. (2004) and Mashburn and Atkinson (2004) did publish ACTH challenge results for Steller sea lions. Relative to ACTH injection, and therefore a perceived stressor, the times between 60 and 95 min for serum cortisol (Mashburn and Atkinson, 2004) and 28–40 hr for fecal corticosterone (Hunt et al., 2004; Mashburn and Atkinson, 2004) show the ''peak'' stress hormone concentration. The close physiological and geographical profiles of Steller and California sea lions allowed us to use the ACTH challenge data of Steller sea lions as a guideline to interpret the California sea lion results of this study.

TABLE 2. Summary description of California sea lion fecal corticosterone concentrations (ng/g dry weight) at time zero (time of procedure; T_0) and the maximum fecal corticosterone concentrations within 48 hr post procedure (max.) for each individual animal in Group A, physical restraint; Group B, inhalant gas anesthesia for non-surgical procedures; Group C, minimally invasive surgery; Group D, invasive surgery

ID#	T_0	Max.	Δ	Admit (A) or pre-release (P)
Group A				
5595	228.1	1,007.3	+	P
5527	290.3	222.7	+	P
5255	5.8	354.1	+	P
5499	498.1	1112.7	+	P
5289	1,019.6	388.1	–	P
6139	17,965.4	37,202.6	+	A
5312	1,636.0	1,110.9	–	A
5319	5,686.5	43,521.7	+	A
5318	5,286.1	28,202.4	+	A
Group B				
5221	514.5	515.8	n.c.	P
6022	1,376.7	8,493.5	+	P
5493	393.1	1,550.5	+	P
5814	421.6	477.2	+	P
5844	682.0	1,153.8	+	P
5270	5,712.0	1,136.7	–	A
5259	3,132.6	6,983.6	+	A
5275	127.9	270.6	+	A
5294	302.9	200.1	–	A
5906	3,942.2	4,461.7	+	A
Group C				
5302	130.6	720.1	+	P
6007	1702.5	368.3	–	P
5278	107.9	405.5	+	P
5292	177.5	14,831.7	+	P
5231	117.2	358.4	+	P
5286	207.4	126.5	–	P
5249	152.3	336.5	+	P
5547	4,743.7	2,193.3	–	A
5556	5,213.7	5,923.9	+	A
5265	169.4	421.8	±	A
Group D				
6018	202.5	1,718.6	+	P
6053	167.3	492.8	+	P
5406	372.9	2,974.6	+	P
6039	248.0	112.4	–	P
6116	1,087.3	96.3	–	P

Note: n.c. indicates no discernible change (<5% difference) in fecal corticosterone concentrations pre-procedure and post procedure. (+) indicates an increase and (–) a decrease in fecal glucocorticoid concentration from T_0.

TABLE 3. Summary description of Steller sea lion fecal corticosterone concentrations (ng/g dry weight) at time zero (time of LHX implantation; T_0) and the maximum fecal corticosterone concentrations within 48 hr post procedure (Max.) for each individual animal that underwent a major invasive surgery: the LHX implantation

ID#	T_0	Max.	Δ
TJ-022	130.9	135.6	n.c.
TJ-023	114.6	n.s.	n.a.
TJ-024	n.s.	n.s.	n.a.
TJ-025	n.s.	n.s.	n.a.
TJ-026	335.6	143.1	−
TJ-027	143.1	322.3	+

Note: n.s. indicates no samples were collected within 48 hr post procedure; n.c. indicates no discernable change in fecal corticosterone concentrations pre-procedure and post procedure; n.a. indicates that maximum adrenal output is unable to be determined owing to lack of samples collected at the appropriate time. (+) indicates an increase in adrenal output from T_0 and (−) indicates a decrease in adrenal output from T_0.

Although some individual California sea lions did exhibit a glucocorticoid response to their specific procedure, none of those responses were significant within groups (Table 2). The proportion of animals with increased post-treatment corticosterone levels was comparable for all four groups (Table 2; A: 7 of 9, B: 6 of 10, C: 7 of 10, D: 3 of 5 animals). Glucocorticoid concentrations vary within and among animals, and animals may respond differently based on daily rhythm, season, degree of domestication, sex differences, and population density (Turner and Bagnara, '76; Oki and Atkinson, 2004). Similar results were found with field handling and anesthesia techniques for radio-collar procedures in African wild dogs (*Lycaon pictus*; Creel et al., '97). Glucocorticoids are essential to the anti-inflammatory response (Breazile, '88) and may be released in larger quantities as part of the response to minor injuries in an individual. Non-surgical anesthetic procedures did not provoke a detectable adrenal response in our study animals as measured by fecal corticosterone RIA.

Mean changes within groups and between pre- and post-procedure fecal corticosterone concentrations (Fig. 2) suggest that handling and restraint (Group A) elicit the largest response, whereas absence of physical restraint results in lower response, the least for anesthesia without procedures (Group B). The comparison between minimally invasive (Group C) and invasive (Group D) surgeries suggests that the glucocorticoid response is similar but lower than physical restraint alone. Southern elephant seal pups (*Mirounga leonina*) showed acute serum adrenocortical responses after physical restraint and blood sampling (Engelhard et al., 2002). Steller sea lions under isoflurane anesthesia also had lower serum cortisol concentrations when compared with voluntary and restraint blood collection (Mashburn and Atkinson, 2004).

Of the six LHX Steller sea lions only one exhibited a clear post-treatment increase in fecal corticosterone concentration (Table 3). Four animals showed practically no changes, and one animal exhibited a decline. However, it is possible that maximum response to treatment may have been missed as a result of opportunistic sampling. Peak fecal corticosterone concentrations are expected within 24–48 hr after exposure to a stressor (Mashburn and Atkinson, 2004). Three of the six Steller sea lions could not be comprehensively sampled during this 48 hr window. Nevertheless, when all fully sampled sea lions from both species subjected to the LHX tag implantation procedures are combined, only three of the seven animals had a clear increase in fecal corticosterone concentration in response to implant procedures, the least response of any treatment group within this study.

It appears that capture and husbandry procedures elicit a stronger glucocorticoid response in sea lions than invasive procedures conducted under gas anesthesia, such as implantation of a telemetry device. On average, maximum post-implant fecal corticosterone concentrations of all seven fully sampled LHX implant recipients (431.6 ± 221.3 ng/g dry weight) were lower than mean pre-procedure levels of any of the four treatment groups A–D (Fig. 2). Of all the fecal samples ($n = 116$) collected from the Steller sea lions, only 23 were considered elevated above baseline levels (> 500 ng/g; Mashburn and Atkinson, 2004; Petrauskas et al., 2006). Furthermore, only eight samples were above 1,000 ng/g (Fig. 3), which corresponded to a decrease in appetite, swollen biopsy site (from a separate earlier sampling procedure), lethargy, and behavior abnormalities such as charging husbandry staff and climbing and pushing fencing. None of the concentrations greater than 500 ng/g appeared to occur in response to a sampling event or LHX implantation procedure. Serum cortisol values further support our interpretation that capture and husbandry without anesthesia elicit the strongest stress response (Fig. 4). In Steller sea lions, the maximum value 7 d post LHX was still lower than all capture values, within 1 month

post-LHX surgery and serum cortisol values are lower than pre-LHX surgery values.

Fecal samples are easily collected and provide an integrated response that represents longitudinal data over a period of time (Möstl and Palme, 2002). Fecal corticosterone analysis is a more practical method to non-invasively monitor the stress response to handling, as well as varying surgical and non-surgical techniques in both California and Steller sea lions. Our results indicate that invasive procedures, under proper veterinary and anesthetic care, do not elicit a consistent significant glucocorticoid response in California or Steller sea lions.

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