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Variation of Fecal Corticosterone Concentrations in Captive Steller Sea Lions (*Eumetopias jubatus*) in Relation to Season and Behavior

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Abstract

Little information is available regarding the adrenal activity of Steller sea lions (*Eumetopias jubatus*) in relation to season and behavior. The objective of this study was to test for seasonal changes in fecal corticosterone concentrations and potential relationships to behavioral scoring in captive Steller sea lions. For this study, fecal samples were obtained opportunistically over a 3-y period (September 2001 to September 2004) from three adult (1 male, 2 female), reproductively intact, long-term captive Steller sea lions housed at the Alaska SeaLife Center in Seward, Alaska. Daily behavior scores based on a scale of 1 (poor) to 5 (excellent) of appetite, energy, attention, sociability, and enrichment were also recorded. The male (SSL-01) had a significantly higher fecal corticosterone concentration in the breeding season, while one of the females (SSL-03) had a significantly higher fecal corticosterone concentration in between the molting and breeding seasons. Fecal corticosterone concentration was significantly higher for one of the females (SSL-03) in comparison to the other female (SSL-02; p □ 0.001). There was a significant negative relationship between behavior score and fecal corticosterone concentrations for the male and one female (SSL-03). The results of this study do indicate that Steller sea lions have a highly seasonal physiology that can be reflected in the fecal corticosterone concentrations of both sexes.

Key Words: Steller sea lion, *Eumetopias jubatus*, corticosterone, stress, season, behavior

Introduction

Stress physiology is the study of perturbations that upset the physiological balance and the mechanisms that re-establish homeostasis in the body. Possible stressors to an organism can commonly include changes in environment or food resources, season, social status, and behavior. A stressor can cause an acute or chronic stress response.

Glucocorticoids are involved in the general homeostasis of energy as well as in acute and chronic stress responses (Kenagy & Place, 2000), but they may fluctuate as a function of season (Oki & Atkinson, 2004). Extreme weather conditions can disrupt breeding if animals are unable to cope physiologically (Romero et al., 2000). Fecal cortisol concentrations from male muriquis (*Brachyteles arachnoids*) were elevated during copulatory periods that coincided with the normal dry season that followed an unusually heavy rainy season (Strier et al., 1999). Season and reproductive state also influence glucocorticoid levels. Male Arctic passerines (*Zonotrichia leucophrys gambelii*) had higher plasma glucocorticoids than females during the breeding season (Astheimer et al., 1994). Corticosterone in breeding Arctic passerines and redpolls (*Acanthis flammea*) of both sexes were significantly higher than their non-breeding counterparts (Astheimer et al., 1994; Wingfield et al., 1994). Plasma corticosterone in male chipmunks (*Tamias amoenus*) peaked immediately following the mating period (Place & Kenagy, 2000), and plasma cortisol changed with the breeding and molting seasons of wild harbor seals (*Phoca vitulina*) (Gardiner & Hall, 1997). Seasons do not appear to influence serum cortisol concentrations in wild and semi-domesticated bottlenose dolphins (*Tursiops truncatus*) (St. Aubin et al., 1996) or in captive harbor seals (*Phoca vitulin*a) (Gardiner & Hall, 1997), although Oki & Atkinson (2004) did find that the diurnal pattern of cortisol secretion was abandoned in the summer in captive harbor seals.

Behavioral endocrinology is the study of how the general physiology of hormones may alter behavior by increasing the chance that a certain behavior will occur when a particular stimulus is present, and in return, how behavior can influence hormones. Behavior is most commonly thought of as an output or a response to a stressor, but the reciprocal can occur; behavior can affect hormone levels. Primates and humans who have lost battles, from territorial contests to chess matches, have

shown reduced testosterone levels immediately following those losses (Nelson, 2000). Accessibility to a variety of toy and feeding enrichment activities positively influenced the behavioral and physiological responses in brown capuchins (*Cebus paella*). An increase in normal behaviors corresponded to a decrease in plasma cortisol (Boinski et al., 1999). Unpredictable events, like severe storms, increased secretion of corticosterone in white-crowned sparrows (*Zonotrichia leucophrys*), which influenced their behavior through altered migration patterns (Wingfield & Ramenofsky, 1997). Rhesus monkeys (*Macaca mulatto*) that did not have control, or lost control, over high intensity noise had significantly increased plasma cortisol levels, increased aggressive behavior, and less social contact with other animals (Hanson et al., 1976). Rats (*Rattus norvegicus*) that could not predict electric shock treatments through a signal exhibited stress-induced pathologies, such as stomach ulcerations, increased plasma corticosterone, decreased body weight, decreased food and water intake, and increased defecation rates, whereas the rats that could predict the shock did not (Weiss, 1970).

In recent years, basic metabolic, physiologic, and veterinary information has been published for Steller sea lions (Rea et al., 1998; Sato et al., 1998; Hunt et al., 2004; Mashburn & Atkinson, 2004; Petrauskas et al., 2006). One study monitored the impact of rehabilitation on growth and fecal corticosterone concentrations for a Steller sea lion pup (Petrauskas et al., 2006). Another study validated fecal corticosterone as a method to monitor adrenal activity in Steller sea lions (Mashburn & Atkinson, 2004). Little information is available regarding adrenal activity in relation to season and behavior in large marine mammals, however. The primary objective of this study was to monitor the fecal corticosterone concentration in captive Steller sea lions in relation to season and behavior. Monitoring adrenal activity in captive Steller sea lions may provide a control or baseline for free-ranging studies due to the copious amount of known fecal samples that can be collected from animals housed in a stable environment. These fecal corticosterone concentrations, in turn, can be correlated to the season and to the age, sex, and behavior of the animal.

Materials and Methods

Fecal samples were obtained from three adult (1 male, SSL-01; 2 females, SSL-02 & SSL-03), reproductively intact, captive Steller sea lions housed under ambient conditions at the Alaska SeaLife Center (ASLC) in Seward, Alaska. All animals were 8 y of age at the beginning of the sample collection. Behavioral scoring on a scale of 1 to 5 (Table 1), with 5 classified as exceptional and 1 as poor, was obtained by calculating the weekly mean score of appetite, energy, attention, sociability, and enrichment by the marine mammal husbandry staff. Behavioral data were collected during each session worked with an individual sea lion, on average 2 to 4 times per day. Samples were collected opportunistically from September 2001 through September 2004 and frozen at -20° C until extraction.

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 (± 0.001) was weighed and extracted as previously described (Monfort et al., 1996; Mashburn & Atkinson, 2004; Petrauskas et al., 2006). Methanol extractant (100 ml) was aliquoted into polypropylene tubes, dried under forced air, and reconstituted in 400-ml buffer for a final 1:4 dilution. Sample dilutions were stored frozen at -20° C until radioimmunoassay (RIA).

A double antibody RIA kit (MP Biomedicals, Costa Mesa, CA), previously validated for use with Steller sea lion fecal extracts (Mashburn & Atkinson, 2004), was used for corticosterone analysis. Standard curves of RIAs were log-logit transformed in order to read the hormone concentrations off the standard curve (Rodbard, 1974). Values from the RIA were corrected for dilution,

Table 1. Behavioral scoring criteria used by the marine mammal husbandry department at the Alaska SeaLife Center to quantify how productive a session was with respect to session goals

Score	Appetite	Energy	Attention	Sociability	Enrichment
1	Uninterested in food	Low	Low	Aggressive to trainers & other animals	Not engaging
2	Little	Slow	Slow	Somewhat interested	Little engagement
3	Normal	Average	Good	More interested	More engagement
4	Eating well	Good	High	Interactive	Engaging
5	Eating very well	Exceptional	Exceptional	Very interactive	Very engaged

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 less than 0.01% for all other steroids tested. Interassay coefficients of variation for two separate assay controls were 14.9 and 14.5%, respectively ($n = 10$ for all samples). Intra-assay coefficients of variation were < 5%, and assay sensitivity was 12.7 ng/tube.

Data Analysis

A repeated measures design was used to determine if the difference between season and behavior was related to corticosterone concentrations. Fecal corticosterone and time of year was tested for significance using the linear-circular rank-correlation test. Differences in corticosterone concentration and social status were tested using the Mann-Whitney rank sum test. Linear regression with one-way analysis of variance (ANOVA) and the Mann-Whitney rank sum test were used to test the relationship between behavior and corticosterone concentration. Significance was based at a P-value □ 0.05.

Results

There was a significant relationship between Julian day and fecal corticosterone concentration for SSL-01 ($p < 0.01$) and SSL-03 ($p < 0.05$) (Figure 1).

There was a significant negative linear relationship between behavior score and fecal corticosterone concentration for the male (SSL-01: $p = 0.0007$; Figure 2a) and one of the females (SSL-03: $p = 0.0383$; Figure 2c), as fecal corticosterone concentration increased, the behavior score decreased. There was no significant relationship for the other female (SSL-02; Figure 2b). There was a significant difference between the weekly mean behavior score (appetite, attention, energy, sociability, and enrichment) of animals with elevated fecal (≥ 500 ng/g) corticosterone concentrations and non-elevated (□ 500 ng/g) concentrations. SSL-01 ($p = 0.002$; Table 2) exhibited increased behavior scores in conjunction with non-elevated fecal corticosterone concentrations, while SSL-02 ($p = 0.013$; Table 2) produced increased behavior scores in conjunction with elevated fecal corticosterone concentrations.

In this study, SSL-03 had significantly higher fecal corticosterone concentrations than SSL-02 ($p = 0.001$).

Discussion

Fecal corticosterone concentrations varied with season and behavior, with individual seasonal patterns in two of the three Steller sea lions. The breeding season of Steller sea lions begins in May when males return to the rookery to defend and maintain prime breeding sites (Maniscalco et al., 2006). Reproductive condition was based on season, and it has been well-documented that glucocorticoids and reproductive hormones vary based on reproductive status (Astheimer et al., 1994; Wingfield et al., 1994; Theodorou & Atkinson, 1998; Strier et al., 1999; Place & Kenagy, 2000). The male sea lion in the present study had the highest corticosterone concentrations in the early part of the breeding season into the height of the season. The captive female Steller sea lions at ASLC began their estrus in July of all study years as indicated by a pink and swollen vulva. Elevated corticosterone levels for the females were just prior to the molt for SSL-02 and at the end of winter for SSL-03, which is outside of the breeding and molting season. The gender difference may be attributed to the increased adrenal output required by males in the breeding season to defend territories and mate with females. Prior to molt and during the winter season, females, who are smaller in size and blubber mass, may require additional energy that the gluconeogenesis from increased corticoids provides.

Behavior was based on the mean of appetite, energy, attention, sociability, and enrichment scores in the husbandry record. SSL-01 and SSL-03 displayed decreased behavior scores with increased fecal corticosterone, indicating that increased corticosterone was associated with degenerative or poor behaviors in response to training/husbandry. Immediately prior to and during the breeding season, male Steller sea lions are engulfed in territorial battles that would potentially decrease the behavioral scoring in the categories of appetite, attention, sociability, and enrichment. Typically, female Steller sea lions do not engage in the same territorial battles as the males, but they do have to cope with perceived stressors on the rookery such as the territorial battles going on around them or weather and surf conditions. SSL-02, the subordinate female, was the opposite of the male and dominant female. As the fecal corticosterone concentrations became elevated, the behavior scores were increasing. This indicated that SSL-02's energy, appetite, socialization, enrichment, and attention were better when her adrenal glands were producing higher amounts of corticoids. As the subordinate, SSL-02 may require more energy when interacting with the husbandry staff and for maintaining awareness of the dominant female or the male.

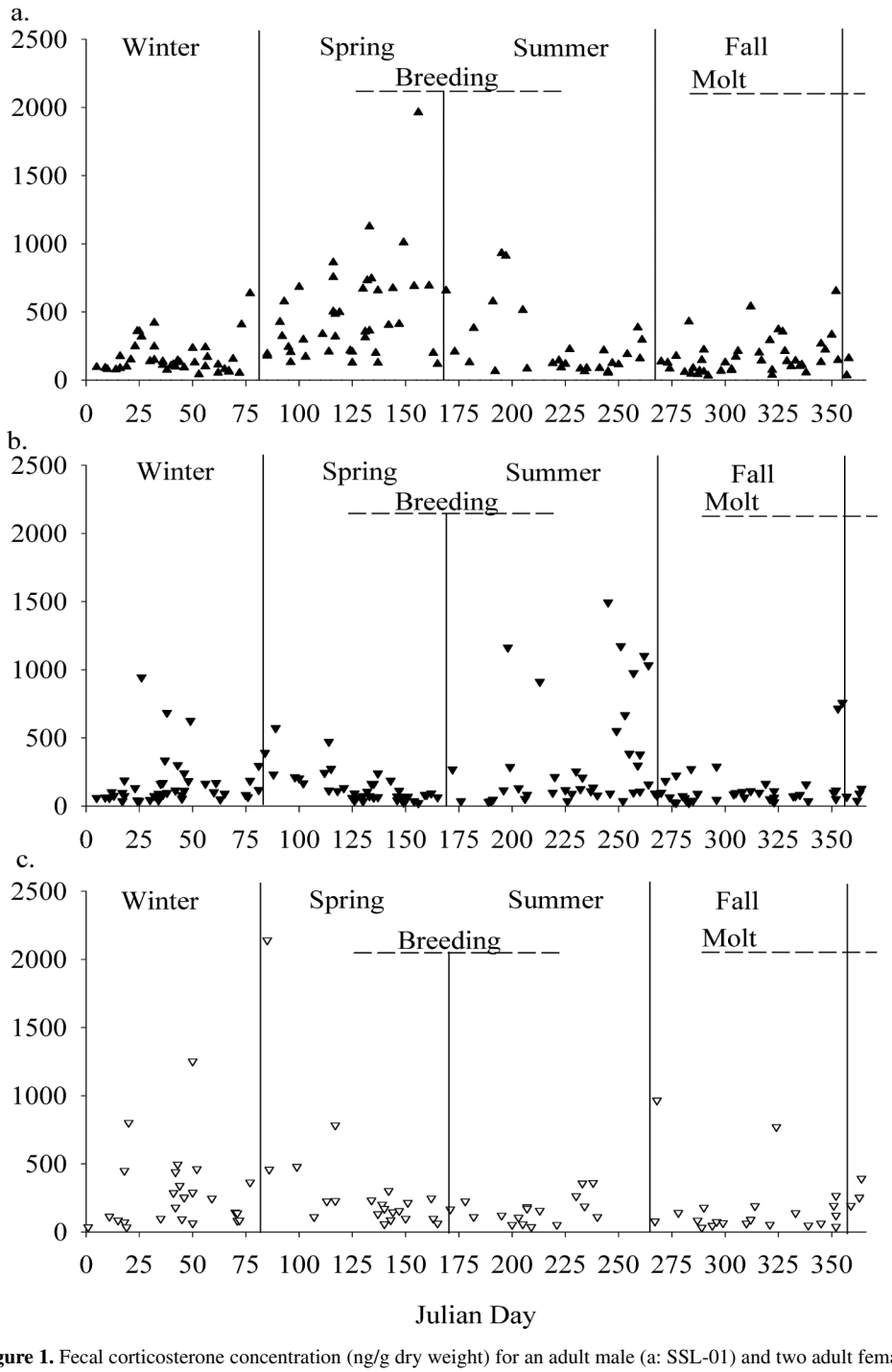

Figure 1. Fecal corticosterone concentration (ng/g dry weight) for an adult male (a: SSL-01) and two adult female (b: SSL-02 and c: SSL-03) Steller sea lions from September 2001 to September 2004 based on Julian day; all years were combined and presented.

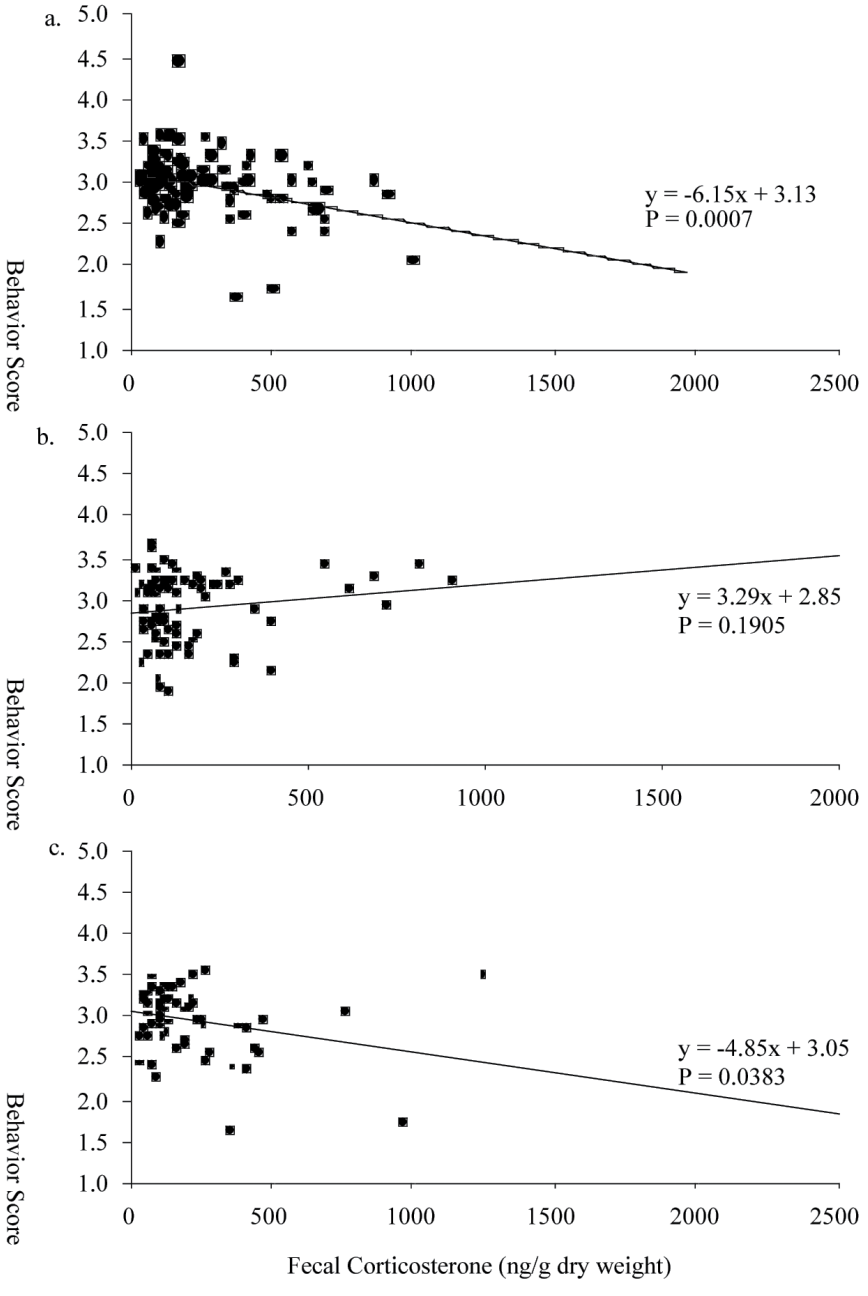

Figure 2. Linear regression and analysis of variance (ANOVA) for one adult male (a: SSL-01) and two adult female (b: SSL-02 and c: SSL-03) Steller sea lions for mean weekly behavior score and fecal corticosterone concentrations for samples collected from September 2001 to September 2004

Agonistic and aggressive interactions among social and cooperatively breeding species can be a chronic stressor (Sands & Creel, 2004). In this study, SSL-03 typically displaced SSL-02 by physically pushing her out of the way or approaching the area of SSL-02, who then departed. SSL-03 has also been observed to steal food from SSL-02. Fecal corticosterone concentrations for the dominant female (SSL-03) were significantly different than those of the subordinate female (SSL-02).

Table 2. Mean behavior score during periods of elevated (≥ 500 ng/g) and non-elevated (□ 500 ng/g) fecal corticosterone concentrations for one adult male (SSL-01) and two adult female (SSL-02 & SSL-03) Steller sea lions; significance tested using the Mann-Whitney rank sum test.

	Elevated	Non-elevated	*T*-value	*p*
SSL-01	2.6 ± 0.48	3.0 ± 0.33	520.5	0.002
	n = 16	*n* = 94		
SSL-02	3.2 ± 0.17	2.8 ± 0.41	446.5	0.013
	n = 7	*n* = 76		
SSL-03	2.8 ± 0.96	2.9 ± 0.37	113.5	0.622
	n = 3	*n* = 61		

The highest measured fecal corticosterone concentrations for SSL-03 corresponded with training sessions that included interactions with the subordinate female. Early studies, mainly with rats and mice, suggested that subordination was stressful (Bronson & Eleftheriou, 1964; Louch & Higginbotham, 1967; Bronson, 1973). The territorial behavior of Weddell seals (*Leptonychotes weddellii*) during the breeding season showed that the dominant males had higher serum cortisol concentrations than subordinate males (Bartsh et al., 1992). Wild African wild dog (*Lycaon pictus*), dwarfed mongoose (*Helogale parvula*), and wolf (*Canis lupus*) studies also suggest that chronic stress is apparently a cost of social dominance rather than subordination (Creel & Creel, 1996; Sands & Creel, 2004).

It is important to identify if sex, social status, or season influence, or are influenced by, adrenal hormones or their metabolites. The small sample size only allowed the identification of a correlation of season and behavior in one male and two female Steller sea lions. Only the male and the dominant female exhibited a significant seasonal correlation with fecal corticosterone concentrations, where the male displayed significantly higher corticoid concentrations during the breeding season and the dominant female displayed significantly higher fecal corticoids during the non-breeding/non-molting seasons. Both the male and the dominant female displayed a significant negative correlation between behavior score and corticosterone concentrations. Despite the small sample size, these results indicate that, like other pinnipeds, Steller sea lions have a highly seasonal physiology that can be reflected in the fecal corticosterone concentrations of both sexes. Fecal samples can be collected easily in the field year-round to monitor the adrenal health of Steller sea lions. Future studies to increase the sample size for each sex as well as to increase the age distribution will provide a critical baseline as a monitoring tool for samples collected from free-ranging Steller sea lions.

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