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Variability of Hormonal Stress Markers and Stress Responses in a Large Cross-Sectional Sample of Elephant Seals

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LONG-TERM GOALS

Physiological indicators of stress in wild marine mammals, the interrelationships between different stress markers and assessment of the biological effects of stress can be used to estimate the impact of anthropogenic stressors on marine mammal populations. Currently, there are no large cross-sectional datasets of stress markers in free ranging marine mammal populations. Without these data there is no context with which to interpret the biological significance of variation in stress markers in individuals. The United States Navy, as part of its environmental stewardship, can utilize stress markers to assess the acute and chronic impacts that its actions might have on marine mammals. This approach would permit better mitigation of potential impacts and ensure that Navy activities do not come at a deleterious cost to marine mammal populations.

OBJECTIVES

The objectives of this effort are to: 1) determine the variation in glucocorticoid hormones (GC), aldosterone (A), thyroid hormones (TH), and catecholamines within a free-ranging northern elephant seal population and its dependence upon gender, age, seasonality, time of day, reproductive state and fasting duration; 2) establish relationships between serum GC levels and levels found in fur and blubber; 3) perform adrenocorticotropic hormone (ACTH) and thyroid stimulating hormone (TSH) challenges and characterize the activation of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-thyroid (HPT) axes across multiple matrices.

APPROACH

Task 1 – Natural variations in hormones across multiple matrices
Baseline characterization of hormones will be conducted during all four years (36 months) of the study with the highest effort in the first year. We will obtain 260 matched blood, blubber and fur samples in year one. We will obtain samples from 80 known-aged females and 60 adult males, either early or late in their natural fasts during the breeding or molt haulouts. Similarly, we will sample 80 known-age juvenile seals early or late in either of their haulouts and 40 weaned pups early and late in their post-weaning developmental fast. During year two of the study we will sample 40 known age adult females, 40 adult males, 40 known age juveniles and 40 weaned pups early and late in the haulout as above. We will target sampling times to complete a broad sample for assessing diel variation. During year three of the study we will repeat the process. The complete effort will achieve a total sample size of 580 individuals. As a value added component of the study we will obtain blood samples only for analysis of stress hormones from animals involved in other ongoing elephant seal research projects (approximately 100-150 additional individuals per year).

Serum samples will be processed for ACTH, cortisol, aldosterone, catecholamines (epinephrine, norepinephrine), and TH (T3 and T4) via radioimmunoassay (RIA). All of these hormones are measured routinely in our lab and the assays have been validated for use in this species. Field collected samples for catecholamine analysis will be centrifuged on site and immediately frozen in liquid nitrogen prior to transport. A multi-step biphasic organic solvent extraction will be used to isolate the corticosteroids from the blubber tissue (Kellar *et al.*, 2006; Kellar *et al.*, 2009). The hormones will be measured using a commercially available enzyme immunoassay (EIA) and parallel processed via HPLC to verify method performance. Hair samples will be collected from the anterior back region of seals for determination of cortisol as a measure of chronic stress (Davenport et al., 2006). Hair shaft cortisol will be determined using a technique recently validated for use in free-ranging terrestrial mammals (Macbeth et al., 2010). Briefly, hair samples will be washed to remove external contamination and then ground to a fine powder with a mixer mill. Cortisol will be extracted into methanol, reconstituted in phosphate buffer, and measured using a commercially available enzyme-linked immunosorbent assay (Macbeth et al., 2010). Blood chemistries will be run for all individuals as part of a health assessment at the time of sampling

Task 2 – Adrenocortical sensitivity and temporal pattern in matrices.
Adrenocortical sensitivity and the relationship between activation of the HPA axis and reflection of this activation in serum and other matrices will be determined during second and third years of the study. In year two, we will complete pilot work for the ACTH, cortisol and TSH studies, working out appropriate dosages for the challenge work in year 3. We will complete 4-6 ACTH and cortisol challenges early and late in the fasts in yearlings and breeding adult females. Initial baseline samples

will be taken for all challenges. ACTH slow-release gel will be intramuscularly implanted to permit time-controlled and sustained release of ACTH. Repeat samples will be taken daily to determine the relationship between the time course of serum GC increase and ACTH dose. The number of days over which sampling will occur will depend on the results of pilot work conducted during the second year of the study. Serum, blubber and fur samples will be processed as described in Task 1.

Direct cortisol challenge will be used to raise cortisol levels independent of HPA axis up-regulation and to potentially allow elevation of cortisol if adrenocortical sensitivity is reduced. Furthermore, the time course of the ACTH challenge may be insufficient for serum cortisol elevations to be observed in the blubber. Therefore, in year 2 small cortisol implants that release cortisol over 21 days will be implanted in 2 weaned pups and 2 juveniles. Two dose rates will be used for the pups, with the same dose rates used for the females. Blood samples will be taken 0, 12, 14 and 16 days after implantation for plasma cortisol measurements, and blubber samples will be taken at 12 and 16 days after implantation for blubber cortisol measurements. In year 3 we will perform ACTH challenges in 10 weaned pups and in 10 juveniles. We will perform cortisol implant challenges in 5 weaned pups and 5 juveniles.

Task 3 – TSH challenges
Thyroid hormones (thyroxin, T4 and triiodothyronine, T3) are released from the thyroid gland and are responsible for regulating a number of metabolic functions, including the regulation of catecholamine activity through permissiveness. Individuals will receive an intramuscular injection of thyroid-stimulating hormone, TSH (Genzyme Corporation, Cambridge, MA, USA). Baseline blood samples will be collected prior to the first injection and then every 30 minutes for 4 hrs from the time of injection. We will perform 2-3 pilot challenges in year 2 and challenges in 5 weaned pups and 5 breeding females in year 3.

Task 4 - Biological significance of baseline hormone values.
We will leverage the large existing research effort on northern elephant seals to assess the biological significance of the variation in hormone levels including survival impacts in juveniles and reproductive impacts in adults. Survival and natality at varied hormone levels will be compared to the larger annual samples of juvenile survival and female natality rates. Furthermore, where information on foraging success can be obtained (i.e. returning tagged females), relationships to foraging success and stress markers will be performed. This might be a particularly fruitful area for exploring relationships between energy acquisition and stress, as recent observations suggest that elevated cortisol is correlated with reduced energy gain at sea in adult female elephant seals and that elevated cortisol levels at implantation or during gestation may play an important role in determining natality in a given year. We will examine the relationship of stress responses to reproductive hormones to better undertsand the mechanism by which stress responses may impact reproduction.

WORK COMPLETED

Task 1 – Natural variation in stress hormones across multiple matrices.
We have completed all of the proposed sampling during the first three sampling periods of the project, successfully sampling 120 animals. The next sampling phase commences with the breeding season in December.

Table 1: Completed serum/blubber/hair samples as of 9/30/01

	Early fast	Late fast
Molting adult females	25	25
Molting adult males	15	15
1st Juvenile haul out	20	20

RESULTS

As of this report, analysis of the samples is just beginning. Analyis of hair samples is underway at University of Saskatchewan and analysis of blubber samples is starting at SWFS. Analysis of serum cortisol levels and blood chemistries are underway at SSU, with analysis of the remaining suite of stress hormones anticipated to be completed by the start of the next field sampling season in December.

IMPACT/APPLICATIONS

The ability to identify stress markers and their relationship to the health of marine mammal populations is critical to understanding the impact of anthropogenic activities upon those populations. The baseline characterization of stress marker variation in elephant seals as a function of seasonality, gender, age, fasting duration, health and reproductive status is important to assessing measurements made in wild pinnipeds and other species, including understanding acute natural variation in stress hormones in contrast to sustained stress reponses resulting in biological impacts. Information on levels and dynamics of stress markers between different matrices will provide better estimates of the overall condition of marine mammals sampled in the wild from either blubber biopsies or hair samples. In addition, an understanding of the function of the HPA and HPT axis and variation in axis function across life histories will provide fundamental information on the mechanics of stress responses in these marine mammals, which may differ significantly from that of the terrestrial mammals. An increased understanding of the mechanisms by which stress hormones interact with physiologicval variables and reproductive status will provide critical information to understanding when stress impacts become biologically significant to populations of marine mammals.

RELATED PROJECTS

Project: Variability of Hormonal Stress Markers Collected from a Managed Dolphin Population
PI: Dorian Houser
This project examines variation in stress hormone markers across several matrices in a captive dolphin population, allowing intensive longitudinal sampling in contrast to the broad, cross-sectional sampling of our study.

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