Chapter 56
Natural Variation in Stress Hormones, Comparisons Across Matrices, and Impacts Resulting from Induced Stress in the Bottlenose Dolphin

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Abstract Knowledge regarding stress hormones and how they vary in response to seasonality, gender, age, and reproductive status for any marine mammal is limited. Furthermore, stress hormones may be measured in more than one matrix (e.g., feces, blood, blubber), but the relationships between levels of a given hormone across these matrices are unknown, further complicating the interpretations of hormones measured in samples collected from wild animals. A study is underway to address these issues in a population of bottlenose dolphins trained for voluntary participation in sample collections from different matrices and across season and time of day.

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1 Introduction

The last decade has seen an increase in the number of research efforts attempting to characterize the response of marine mammals to anthropogenic noise exposures. These responses have frequently been investigated using behavioral response studies under the assumption that the disruption of natural behaviors results in some cost to the animal whose behavior has been disrupted. The costs presumably scale with the behavior that is disturbed. Certainly, missed breeding and foraging opportunities can potentially be related to decreased fecundity or reduced energy acquisition and other altered behaviors might have energetic or fitness costs as well. However, the consequences of anthropogenic noise exposure can also occur at physiological levels that might not correlate with behavior, a process that is often referred to as the "stress response."

The concepts of stress and the stress response have evolved from the notions of homeostasis and the general adaptation syndrome (Cannon 1932; Selye 1936). There is no universally accepted definition of stress, and models of stress and the stress response are currently topics of debate (e.g., allostasis vs. the reactive scope mode; Romero et al. 2009; McEwen and Wingfield 2010). Nevertheless, the physiological response to an external or internal perturbation that enables an animal to respond to and recover from the perturbation can be loosely defined as a stress response. The stress response, which is beneficial to an animal over certain magnitudes and timescales, still results in costs to the organism, and remaining in a persistent stressed state can result in disease, reduced fecundity, decreased longevity, and physiological dysfunction.

The general adaptation syndrome is probably the most notable and well-characterized example of the stress response. In short, the general adaptation syndrome states that when an animal is affected by a stressor, the body produces glucocorticoids to prepare the animal for dealing with the stressor. Cortisol, the major glucocorticoid in many mammals, increases blood glucose, affects metabolism of fat and protein, and mediates inflammatory processes. Although cortisol is the most heavily studied of the stress hormones, it is now known that a suite of hormones can help an animal deal with both acute and chronic stressors. These include other glucocorticoids, the catecholamines (epinephrine and norepinephrine), thyroid hormones, and other neuroendocrine responses.

The stress response has received considerable attention in some biological systems, particularly as the field of conservation physiology has developed over the last couple of decades. However, the stress response is poorly understood in sound-exposed marine mammals and is limited by information on stress hormone variability as a function of age, gender, reproductive status, life history stage, and seasonality for nearly every species of marine mammal. This information is critical in interpreting stress hormone measurements obtained from wild marine mammals and made from various matrices (e.g., feces, blood, blubber), particularly because

blubber and feces are more likely candidate matrices for collection from wild animals than is blood. Here, a current study is described in which baseline hormonal variation, relationships between hormone levels in different matrices, and the responsiveness of components of the stress response system are characterized in the bottlenose dolphin (*Tursiops truncatus*).

2 Methods

2.1 Season, Age, and Gender Effects

Thirty *T. truncatus* housed at the US Navy Marine Mammal Program were monitored for a year to characterize seasonal and demographic variations in the corticosteroids (cortisol and aldosterone), catecholamines (epinephrine and norepinephrine), and thyroid hormones and to determine the relationships between hormone levels measured across different matrices. Voluntary blood and fecal samples were collected biweekly through voluntary participation of the subjects. Blood samples were collected between 700 and 1,000 h. Monthly blubber biopsies were obtained from a subset of the subjects using a tissue biopsy punch. Serum and plasma hormones were processed by radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). Plasma catecholamines were processed in parallel with high-performance liquid chromatography (HPLC) as an external validation of RIA methods. Hormone metabolites were extracted from the blubber and fecal samples before processing with RIA.

2.2 Diurnal Variation

A current, year-long study is investigating diurnal variation in hormone levels. Sampling is performed as described in Section 2.1, but only ten animals are being utilized and samples are collected on a monthly basis. The study is methodologically different from the seasonal study in that voluntary blood samples are collected at three different times on the day of collection, at 700, 1,200, and 1,700 h. Fecal samples are collected the day after and 1 week after blood sampling.

2.3 Cortisol Feeding Study

A study was conducted with five *T. truncatus* that were fed cortisol every 6 h over a period of 4–5 days. Blood samples were taken on a daily basis and blubber biopsies were collected every 2 days to determine the relationships between blubber and serum cortisol levels and to characterize the biological half-life of cortisol.

2.4 Adrenocorticotropic Hormone Challenge

Adrenocorticotropic hormone (ACTH) challenges are currently being conducted to investigate the time course of hormone variability across matrices after an induced stress event and to determine immune system impacts resulting from persistently elevated cortisol. Subjects are given an intramuscular injection of ACTH slow-release gel and repeated blood samples are collected over a period of 5 days. Blubber samples are collected every 2 days and fecal samples are collected daily. All matrices will be processed as previously described to characterize activation of the hypothalamus-pituitary-adrenal (HPA) axis and to determine how the cascade of hormone variations is reflected in the different matrices.

2.5 Thyroid Hormone Challenge

Thyroid hormone challenges will be conducted to investigate the time course in hormone variation across matrices following an acute increase in thyroid hormones, which are key regulators of metabolism. Subjects will be given an intramuscular injection of either thyroid-stimulating hormone (TSH) or thyrotropin-releasing hormone (TRH). Blood samples will then be repeatedly collected over a period of 4 h while fecal samples will be collected opportunistically for a period of 96 h after the injection. All matrices will be processed as previously described to characterize activation of the hypothalamus–pituitary–thyroid (HPT) axis and to determine how the cascade of hormone fluctuations are reflected in the different matrices.

3 Results

Samples from the seasonal study and cortisol feeding study are currently being processed. The diurnal variation study and ACTH challenge study are currently underway. The thyroid hormone challenge study is slated to begin later this year. Because each of these studies is in a different stage of progress, only results from preliminary analyses are presented here.

Mixed models were used to evaluate variability across individuals in the seasonal study; an individual subject was included as a random effect to account for repeated sampling, and each hormone was used as a response variable. No significant differences were observed between the sexes for any of the hormones investigated to date ($P > 0.05$ for ACTH, thyroid hormones, and catecholamines). However, several hormones showed significant seasonal variation ($P < 0.05$ for epinephrine, norepinephrine, and thyroid hormones). ACTH and corticosteroid concentrations were markedly lower than those reported for wild-caught animals, but low levels were not due to adrenal exhaustion because certain veterinary procedures were observed to activate the HPA axis and increase both cortisol and aldosterone.

4 Discussion

The study described here is the largest and most comprehensive study of stress hormones in a cetacean; to date, over 1,000 individual serum and plasma samples have been collected representing males and females from 6 to 42 years of age. The results will provide comprehensive information on seasonal, diurnal, and demographic influences on hormone variability in *T. truncatus* as well as on the relationships between hormone levels and fluctuations in different matrices. It will provide a baseline to which measurements in wild dolphins and captive dolphins exposed to anthropogenic sound can be compared and will thus provide a context in which to determine how physiological responses to anthropogenic stressors, including acoustic stressors, might deleteriously impact marine mammals.

The preliminary analysis here suggests that the hormone values observed in *T. truncatus* under human care and collected under voluntary conditions are markedly lower than those reported for wild-caught animals. This suggests that free-ranging *T. truncatus* typically have higher concentrations of ACTH, aldosterone, and cortisol due to environmental factors or that handling disturbance in wild *T. truncatus* increased hormone concentrations before sample collection. Preliminary analyses further suggest that aldosterone production is an important aspect of the stress response in *T. truncatus*. Relative to terrestrial mammals, this may have greater implications for stress impacts on marine mammals because of their life in a hyperosmotic environment. As such, aldosterone should potentially be given the same level of consideration as a stress marker that cortisol has received, particularly because the two hormones share a synthetic pathway.

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