

## **The Bottlenose Dolphin (*Tursiops truncatus*) as a Model to Understand Variation in Stress and Reproductive Hormone Measures in Relation to Sampling Matrix, Demographics, and Environmental Factors**

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### **LONG-TERM GOALS**

Our overarching goal is to develop indicators and methods to quantify chronic stress in bottlenose dolphins. Much research has focused on the stimuli which induce stress in marine mammals, as well as the hormonal mediators of the stress response. Stress may be induced by a variety of factors, including noise, pollutant or toxin exposure, presence of predators, loss of prey and/or habitat changes. The stress response is complex and difficult to study experimentally in marine mammals due to ethical and logistical considerations, but has been well characterized in other laboratory mammal species. In mammals, as well as other vertebrates, the stress response has two modes of operation. The fast mode involves the rapid release of fast-acting agents such as catecholamines by the medulla which drives the fight-or-flight response, enhancing vigilance, alertness, arousal and attention. The catecholamines in turn play a major role in excitation of the hypothalamic-pituitary-adrenal (HPA) axis, initiating a hormonal cascade which culminates in stimulation of the adrenal cortex to secrete glucocorticoids (GCs). The delayed but more sustained response driven by GCs coordinates brain and body functions to cope with stress and facilitate recovery, adaptation and re-establishment of homeostasis. These functions include mobilization of substrates for energy metabolism, suppression of immune and inflammatory reactions and inhibition of bone and muscle growth. Studies of both captive and free-ranging individuals support the existence of these same stress response pathways in marine mammals.

While the HPA axis and physiological processes driven by GCs are essential for an individual's ability to respond and adapt to stress, prolonged stimulation can overly burden the body's regulatory systems and induce deleterious effects. Prolonged elevation of GC hormones can lead to chronic immune suppression and inhibition of other energy-expending hormonal systems, including disruption of reproductive function along the hypothalamo-pituitary-gonadal axis, all of which may cumulatively lead to decreased survival and/or inability to reproduce. For this reason, developing indicators and methods to quantify chronic stress in marine mammals is essential for understanding risks and long-term consequences for populations.

# Report Documentation Page

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## OBJECTIVES

Using the bottlenose dolphin as a model species, specific objectives for this project are:

- Determine correlation of hormone measures (cortisol, T3, T4, FT4, reproductive hormones) between blood and blubber
- Develop a comprehensive understanding of factors that influence stress hormone levels and establish reference intervals for blood and blubber measurements, determining necessary stratifications by sex, age, and/or sampling season
- Examine relationships among the various hormone measures and conduct preliminary screening analysis to examine potential relationships between the stress hormones and other health measures including immune function

## APPROACH

The challenge of dealing with free-ranging marine mammals can be reduced through selection of species for which a broad base of biological information is already available and situations where appropriate samples can be readily obtained. Long-term studies of resident populations of well-known species such as common bottlenose dolphins (*Tursiops truncatus*) can help to promote an understanding of the natural variation in hormones and/or biomarkers of the stress response in free-ranging marine mammals as it relates to life history or natural cycles.

Recent work by our collaborative team has supported the use of blubber as a sampling matrix for measuring hormone concentrations (Kellar et al. 2006; Kellar et al. 2009). The dynamics of blubber composition are slower and somewhat buffered from changes in blood, such that the hormone signals trapped with this lipid-laden tissue represent longer windows of physiological time and are not heavily influenced by the events immediately preceding sampling. Blubber as a sampling matrix provides the additional advantage that samples can be readily obtained using remote biopsy, which is relatively inexpensive and entails less harassment compared to capture-release sampling. In addition, while the logistics of capture-release sampling generally limit its utility to investigations of coastal cetaceans, remote biopsy has the potential to be a powerful tool for investigations across a range of habitats, from estuarine to nearshore and pelagic populations.

Preliminary indications from a pilot study using a small number of matched samples from live bottlenose dolphins suggest good correlation between blood and blubber measures of progesterone, and pregnant individuals were easily identified using either serum or blood concentration. Analyses of the same samples for GCs (cortisol and corticosterone) are pending, but given the strong similarities in structure and chemistry between all steroid hormones, it is very likely that GCs will exhibit similar measurement and diagnostic capabilities to these more well-studied reproductive steroid hormones. In addition to the GCs, we are working to validate the measurement of thyroid hormone concentrations in the blubber, but this still requires validation of the assay as well as analysis of the correlation between blood and blubber. The ability to measure thyroid hormones from remote biopsy samples will be a huge benefit as recent studies have indicated that health impacts (*e.g.*, immune function and growth) of chronic stress from chemical contaminant exposure may be mediated through the thyroid hormones (Schwacke et al. 2011).

Remote biopsy samples will be collected to obtain sufficient sample sizes, to acquire samples across 4 seasons and to ensure derived reference intervals are applicable across geographic sampling sites. Additional remote biopsy sampling will be conducted across all 4 seasons in estuarine and coastal waters near Charleston, South Carolina, and across 2 seasons in the Ashepoo, Combahee and Edisto (ACE) Basin, also in South Carolina. We define seasons as: Winter (December-February), Spring (March-May), Summer (June-August), and Fall (September-November). Biopsy sampling period will be scheduled opportunistically to take advantage of favorable weather conditions.

The Charleston site is home to a resident community of bottlenose dolphins that has been studied since 1994; these dolphins are one of the northernmost year-round resident stocks on the U.S. coast (Speakman et al. 2010; Zolman 2002). Additionally, since 1997, over 200 remote biopsy samples have been collected from Charleston dolphins, with no adverse impacts. Selection of this northern stock should enable identification of seasonal variation in hormone concentrations, if such variation exists. The ACE Basin site was selected as an additional site for estimation of reference intervals because it is an undeveloped area, home to a National Estuarine Research Reserve and due to its proximity to the NOAA Charleston Laboratory.

We have recently initiated studies targeting populations in heavily impacted coastal sites to gain an understanding of the effects of biological and chemical stressors on dolphin population health. Capture-release studies have been conducted in the Florida Panhandle where we are investigating the effects of chronic algal toxin exposure, in southeast Georgia where we are examining the impacts of high exposure to legacy chemical contaminants and in Barataria Bay, Louisiana where we are studying potential health effects from petroleum exposure and associated chemical contaminants resultant from the Deepwater Horizon oil spill. In all of these capture-release projects, we have collected data on reproductive, and thyroid hormones, as well as indicators of functional immunity (e.g. lymphocyte proliferation and neutrophil and monocyte phagocytosis), all measured simultaneously from the same individuals and processed by the same laboratories to ensure inter-study comparability.

The current project is a collaborative effort, in conjunction with Dr. Nicholas Kellar, NOAA/National Marine Fisheries Service (NMFS), Southwest Fisheries Science Center, Dr. Patricia Rosel, NOAA/NMFS, Southeast Fisheries Science Center, Dr. Stephanie Venn-Watson, National Marine Mammal Foundation and Dr. Teresa Rowles, NOAA/NMFS, Office of Protected Resources. Dr. Wells also serves as PI for a matching project.

## **WORK COMPLETED**

Matched blood and blubber samples have been collected from estuarine dolphins captured, sampled and released from southeast Georgia in August 2009 (n=24) and from Barataria Bay, Louisiana in August 2011 (n=22). Matched blood and blubber samples were collected from dolphins in Sarasota Bay during May 2009 (n=20), May 2010 (n=10) and May 2011 (n=15). More than 2,100 hormone measures previously obtained for Sarasota Bay dolphins will also be applied to this project. Additional samples will be collected in Sarasota Bay during May 2012. In addition, we have begun synthesis of existing blood hormone data collected from previous capture-release projects in St. Joseph Bay, Florida (n=31) and Beaufort, North Carolina (n=16) to incorporate with Sarasota Bay data for calculation of stratified blood hormone reference intervals.

## RESULTS

Remote biopsy sampling near Charleston and in the ACE Basin is scheduled to commence in October 2011.

## IMPACT/APPLICATIONS

We expect to better define the range of natural variability of stress hormones for bottlenose dolphins, as well as stress hormone responses to a variety of natural and anthropogenic stressors. By examining relationships between stress hormones in blood and blubber, we hope to enhance the utility of remote blubber biopsy sampling as a tool for measuring stress hormones, and reduce the need for dolphin capture-release -- a stressful, expensive, and logistically complex activity -- to obtain stress hormone measures. We will also examine potential relationships between stress hormone measures and longer-term dolphin health indicators in order to identify potential impacts of stress.

## RELATED PROJECTS

A matching project is being conducted under the leadership of Dr. Randall Wells of the Chicago Zoological Society (Project No. N000141110542). The current project will provide remote biopsy samples from bottlenose dolphins along the South Carolina coast for examination of seasonal variability in blubber hormone measures. Dr. Wells' study will provide samples and historical hormone and health data from Sarasota Bay bottlenose dolphins. Data analyses are being performed jointly.

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