Assessing Stress Responses in Beaked and Sperm Whales in the Bahamas

Rosalind M. Rolland, D.V.M., Kathleen E. Hunt, Ph.D., & Scott D. Kraus, Ph.D.
John H. Prescott Marine Laboratory
New England Aquarium
Central Wharf
Boston, MA 02110
phone: (617) 973-6587 fax: (617) 973-0242 email: rrolland@neaq.org

Diane E. Claridge, M.Sc. Ph.D. & Charlotte A. Dunn, M.Res.
Bahamas Marine Mammal Research Organization
P.O. Box AB-20714
Marsh Harbour
Abaco, Bahamas
phone: (242) 366-4155 fax: (242) 366-4155 email: dclaridge@bahamaswhales.org

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http://www.bahamaswhales.org

LONG-TERM GOALS

The long-term goal of this project is to develop fecal hormone assays to assess stress responses in Blainville's beaked whales (*Mesoplodon densirostris*) and sperm whales (*Physeter macrocephalus*) inhabiting the northern Bahamas. These deep-diving species were chosen to include a particularly acoustically-sensitive cetacean (beaked whales) and a co-occurring species (sperm whales) for comparison. The immediate goals are to: 1) evaluate the feasibility of fecal sample collection from these two species, 2) validate immunoassays and determine fecal hormone levels for relatively undisturbed reference populations of both species off Great Abaco Island in the Bahamas, and 3) characterize the natural variations in stress-related hormones according to life history stage (age-class, sex, reproductive status). The results of this project will provide baseline levels of these hormones in beaked and sperm whales for comparison with conspecifics inhabiting environments with acoustic exposures, such as the nearby U.S. Navy Atlantic Undersea Test and Evaluation Center (AUTEC), and experiencing known acoustic disturbances including mid-frequency active sonar.

OBJECTIVES

The objectives of the research project in FY2014 were to:

(1) Continue dedicated fecal sampling surveys for Blainville's beaked whales and sperm whales off southwest Great Abaco Island. Fieldwork not completed in FY 2013 because of poor weather conditions was extended into the FY 2014.

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(2) Refine protocols for processing of samples in the field laboratory in The Bahamas to remove seawater shortly after collection. Conduct trials with revised sampling nets to recover more sample.

(3) Process and analyze fecal samples collected in FY 2013 for five steroid and thyroid hormones at the New England Aquarium Endocrinology Laboratory.

(4) Conduct preliminary data analyses.

APPROACH

This project is a collaboration between scientists at the John H. Prescott Marine Laboratory at the New England Aquarium (NEAq; Boston, MA) and the Bahamas Marine Mammal Research Organization (BMMRO; Great Abaco Island, The Bahamas). Sample collection is being led by BMMRO scientists (D. Claridge, C. Dunn) with assistance from NEAq scientists (R. Rolland, S. Kraus, K. Hunt, E. Burgess). Initial sample processing is being conducted in a field laboratory equipped with a centrifuge and basic equipment at BMMRO in the Bahamas. BMMRO is providing individual identification and life history information on sampled whales. Endocrine analyses, data interpretation, project oversight and management, and reporting are being conducted by the NEAq (R.Rolland, K. Hunt, S.Kraus).

The first two years of this project demonstrated the feasibility of the field sampling, and the applicability of the fecal hormone approach for the target species. We successfully validated radio-immunoassays for the fecal metabolites of five fecal hormones for both species, including two stress-related hormones, adrenal glucocorticoids (GCs) and thyroid hormone (tri-iodothyronine, T3), and three reproductive hormones (estrogens, progestins, androgens) (Rolland et al. 2011, 2012). The following years of the project included additional fieldwork for sample collection in The Bahamas to increase sample sizes from whales of different life-stages, setting up a field laboratory for initial sample processing, and a final year to complete laboratory assays of all samples, conduct data analyses and interpretation, write the final project report, and prepare a manuscript.

WORK COMPLETED

Task 1. Sample Collection in the Bahamas

Fieldwork was conducted by BMMRO aboard a 6.8m rigid-hulled inflatable boat off southwest Great Abaco Island. Sample collection was accompanied by photographic documentation of all whales encountered, and images of sampled whales were later compared to existing BMMRO identification catalogues to provide information on the whale's age-class (*i.e.*, calf, juvenile, adult), sex, and reproductive state (mature vs. immature; for females-pregnant, lactating).

Beaked whale samples were collected by towed divers equipped with a small dip-net and a plastic-zipper bag using methods previously described (Rolland et al. 2011, 2013). Sperm whale samples were scooped from the water surface into a net or directly into plastic sample jars. New England Aquarium post-doc, Dr. Elizabeth Burgess, assisted BMMRO with the fieldwork in The Bahamas in June. The beaked whale sample net was revised using a less fine mesh to increase retention of fecal material, but make it easier to remove fecal material from the net mesh (Florida Aquatic Nets, Winter Haven, FL).

For both beaked and sperm whale samples, some seawater was scooped up along with the fecal material. The day of collection, fecal samples were centrifuged, the total volume of seawater was

measured and decanted, and the fecal pellets were frozen separately from an aliquot of the seawater from the same sample (Figure 1). Both sample types will be analysed for hormones at the NEAq laboratory in Option Year 2 of the project. Dr. Burgess conducted several experiments to refine protocols in the field laboratory (at BMMRO) to increase the efficiency of sample processing to remove seawater.

Figure 1. Fecal pellets are visible at the bottom of the Falcon tubes after centrifuging fecal samples from Blainville's beaked whales and removing seawater supernatant.

Task 2a. Hormone Assay Validations
This task was completed in FY 2012 with development of fecal sample processing and hormone extractions protocols for both species, and successful validation of immunoassays for fecal estrogens, progestins, androgens, glucocorticoids and thyroid hormones in both species as previously reported (Rolland et al. 2011).

Task 2b. Hormone Assays
Analysis of all samples collected in FY 2013 was completed in the current year of the project. Samples collected in FY 2014 will be shipped to the NEAq and analyzed during Option Year 2 of the grant (FY 2015).

Task 3. Data Analysis, Publications and Reporting
Preliminary analysis of hormone results from FY 2011 and 2013 has occurred.The majority of analyses will take place in Option Year 2 when additional hormone data from the complete sample set are available.

Results from the first two years of the project were presented orally at two meetings during FY 2014: 1) the Biennial Meeting of the Marine Mammal Society, December 10, 2013 in Dunedin, New Zealand, and 2) The ONR Program Review, May 13, 2014 in Arlington Virginia. Additionally, Dr. Rolland participated at the ONR Acoustic Stress Workshop in Durham, NC, on June 17-18, 2014.

RESULTS

Field Effort

Dedicated sampling surveys were conducted by BMMRO for 19 vessel-days (14.5 full days) aboard a from mid-June to late September 2014. The success of beaked whale sampling, in particular, is dependent upon a Beaufort seastate ≤ 2 and good underwater visibility, and, therefore, days at sea were chosen carefully based on optimal conditions. Surveys were primarily concentrated near the 1000m isobath along the southwest side of Great Abaco Island, in the northern Bahamas (Figure 2). Survey effort totaled 1,472km of vessel track lines. During June, the field team worked with a film crew that covered survey costs for an additional 11 days which increased the number of sperm whale samples collected at no additional cost to the project.

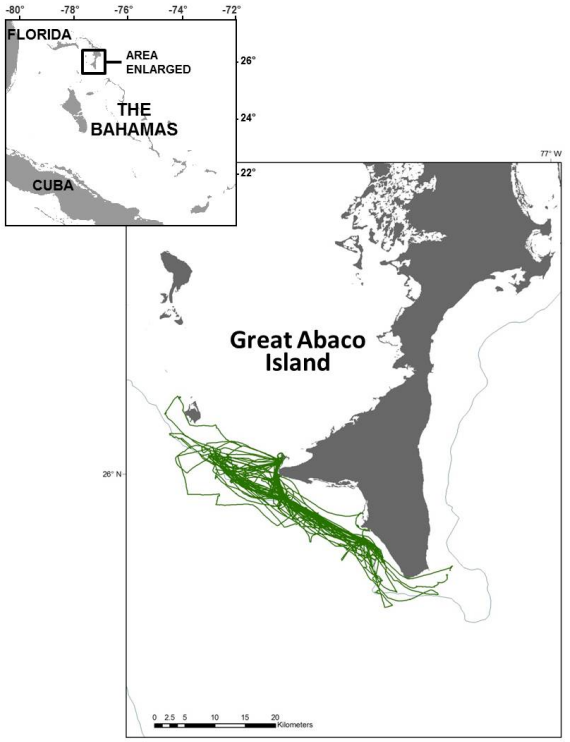

Figure 2. Map showing vessel tracks (green lines) during surveys off the coast of Great Abaco Island during June – September 2014. Survey efforts were concentrated off the southwestern side of the island along the 1000 m isobaths (shown as light grey line).

During surveys, there were 38 cetacean sightings, including seven different species, which included 4 groups of Blainville's beaked whales and 10 groups of sperm whales. Group size ranged from 1-4 whales for Blainville's beaked whales (median = 1) and 1-6 animals for sperm whales (median = 1); notably, group size during the summer 2014 was smaller than previously recorded for both species.

Fecal Sample Collection

All samples were collected off the southwest side of Great Abaco Island (Figure 3).

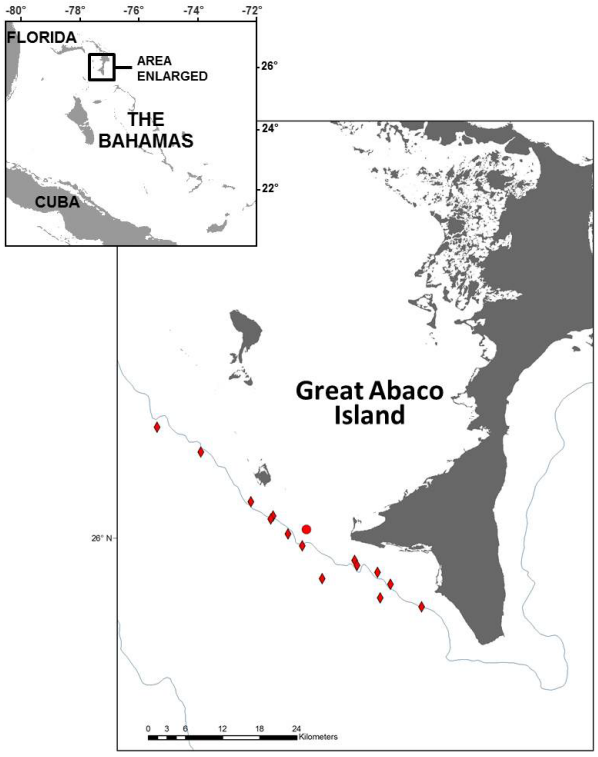

Figure 3. Map showing that fecal samples were collected from throughout the area surveyed off Great Abaco Island during June – September 2014. Blainville's beaked whale sample locations are represented by red circles and sperm whales by red diamonds. The 1000 m line is shown.

Fifteen fecal samples were successfully collected from Blainville's beaked whales (n = 1) and sperm whales (n = 14) during the field effort. Repeated collections were made from the same subadult male sperm whale on seven occasions over 33 days. These samples will be extremely valuable to characterize short-term hormone variation within an individual. Additionally, six control water samples were collected in the vicinity of beaked whales (n = 1) and sperm whales (n = 5). These water samples will be assayed for all five hormones to be sure that there is no background activity contributing to the hormone results.

Collection data including information on the age-class, sex and reproductive status based on individual life histories for known whales are summarized in Table 1. The single beaked whale sample was from an adult male that had not previously been seen in the study area. The majority of sperm whales sampled were matched to the BMMRO catalogue and life history information was available for these individuals.

Table 1. Summary of individual life history data for known sampled whales.

INDIVIDUAL LIFE HISTORY DATA					
Sample #	**Whale ID**	**Sex***	**Age class**	**Reproductive State**	**Notes**
Blainville's beaked whales					
Md 14-01	New whale	Male	Adult	Mature	
Sperm whales					
Pm 14-01	Pm130	Female	Adult	Mature	First seen in 2004
Pm 14-02	New whale	Unknown	Juvenile/Subadult	Immature	
Pm 14-03	Pm053	Unknown	Subadult?	Immature?	First seen in 2000
Pm 14-04	Pm170	Female	Adult	Mature	First seen in 2009
Pm 14-05	New whale	Unknown	Juvenile/Subadult	Immature	
Pm 14-06	New whale	Male?	Subadult?	Immature?	
Pm 14-07	Pm248	Male	Subadult	Immature	First seen in 2013
Pm 14-08	Pm156	Male	Subadult	Immature	First seen in 2007
Pm 14-09	Pm156	Male	Subadult	Immature	
Pm 14-10	Pm156	Male	Subadult	Immature	
Pm 14-11	Pm156	Male	Subadult	Immature	
Pm 14-12	Pm156	Male	Subadult	Immature	
Pm 14-13	Pm156	Male	Subadult	Immature	
Pm 14-14	Pm156	Male	Subadult	Immature	
***If ID is unknown, age and sex class information is from visual observations in the field.**					

Including all fieldwork years (FY2011- 2014), total fecal samples collected for this project to date is 53 including 21 samples from beaked whales and 32 samples from sperm whales (Table 2). Using the custom-made dip nets nets increased collected sample mass significantly. In beaked whales, mean sample mass increased from 0.0743 grams (2011) to 0.2614 grams (2013), and in sperm whales sample mass went from 0.2767 grams (2011) to 1.6982 grams (2013). Based on Dr. Burgess's experiments, the protocols for fecal processing were modified to decrease the time for centrifuging samples by over one-third, greatly speeding up the initial sample processing time.

Table 2. Summary of all fecal samples for this project from Blainville's beaked whales and sperm whales. "Other" refers to samples collected opportunistically by BMMRO during other field projects (2010-2014).

Year	Beaked Whales	Sperm Whales	Total
2011	10	9	19
2013	9	3	12
2014	1	14	14
Other	1	6	7
	21	**32**	**53**

Hormone Assays

Fecal samples of adequate mass (>0.02 g; Hayward et al. 2010) collected to date have had measurable levels of most of the five hormones. Preliminary data analyses for samples collected in 2011-2013 showed expected patterns in most reproductive hormones based on life history stage of identified whales. For example, reproductive hormones in female beaked whales (n=9) vary according to reproductive state, with mature females showing higher progesterone and greatly elevated total estrogens compared to juveniles (Figure 4). Although fewer sperm whale samples were from identified

individuals, several samples had two to three-fold elevations of fecal progestins suggestive of estrous cycles or pregnancy. Thus far only two samples have been collected from adult male beaked whales and none from adult male sperm whales.

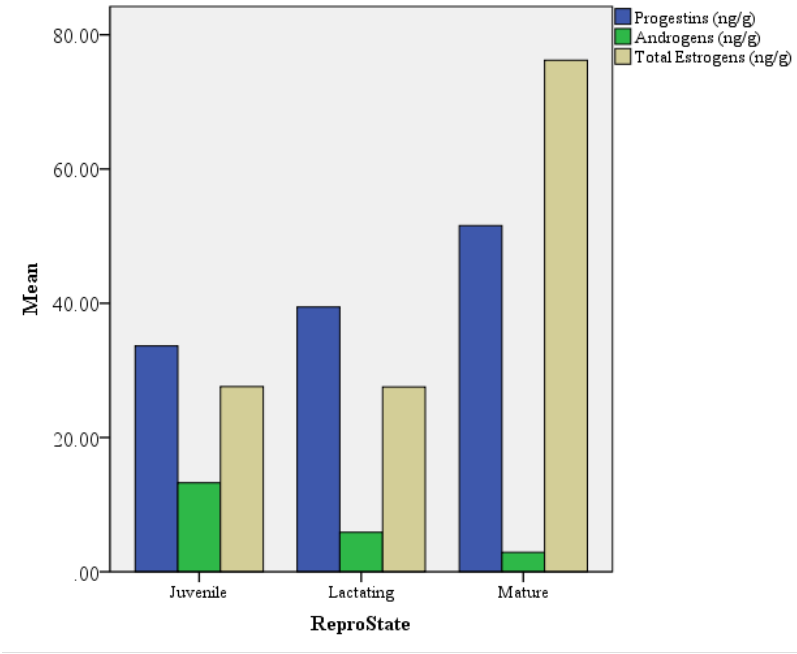

Figure 4. Reproductive hormone metabolites in female Blainville's beaked whales vary according to life stage and reproductive status.

Glucocorticoids were measurable in all samples from both species, and thyroid hormone (T3) was detectable in most samples. Species differences in concentrations of both hormone metabolites were apparent. In beaked whales, glucocorticoids had a fairly tight range of values (Table 3), while three whales had elevated thyroid hormone compared to the mean values, including an adult male, an adult female and a calf. Elevations in thyroid hormone reflect higher metabolic rates that may be related to nutritional state, energetic demand, increased activity or response to thermal changes. In contrast, sperm whales had relatively tight ranges for thyroid hormone, and more variability in GCs (Table 3) in a few whales. Further analysis of hormone results will occur after assay of the 2014 samples.

Table 3. Mean and 95% confidence intervals (CI) for fecal glucocorticoids and thyroid hormones (T3) in Blainville's beaked whales and sperm whales sampled in the northern Bahamas.

	Glucocorticoids (ng/g)			**Thyroid hormones (ng/g)**		
	n	**mean**	**95% CI**	**n**	**mean**	**95% CI**
Beaked whale	13	18.4	12.8, 26.0	13	45.2	14.4, 76.0
Sperm whale	15	54.2	41.9, 66.5	15	13.7	10.1, 17.3

IMPACT/APPLICATIONS

Based on several incidents of mass stranding coinciding temporally and spatially with naval exercises utilizing mid-frequency active sonar (MFAS), beaked whales appear to be particularly sensitive to this type of acoustic exposure (Balcomb & Claridge 2001; D'Amico et al. 2009). Aside from stranding mortalities, it is unknown whether periodic and/or repetitive MFAS events and/or other types of anthropogenic noise lead to chronic stress responses in exposed whales, which could lead to negative consequences for individual or population health (NRC 2005). Therefore, developing methods to better understand the sub-lethal, physiologic consequences of underwater noise disturbance on species of concern, like beaked whales, is crucial to evaluate the potential for long-term impacts of naval exercises and other oceanic activities. The results of this research project have shown that it is feasible to collect fecal samples from both species, and that analysis of stress-related fecal hormones can be successfully applied in both beaked and sperm whales. Although collecting samples from beaked whales, in particular, is challenging, given enough dedicated field effort baseline ranges of stress-related fecal hormones can be developed and used to assess physiologic responses in beaked whales in environments with elevated acoustic exposures from naval activities.

RELATED PROJECTS

The New England Aquarium's Ocean Health and Marine Stress Program includes a related ONR-funded project on *Development of Novel Noninvasive Methods of Stress Assessment in Baleen Whales* (K. Hunt, PI; ONR #N000141310639). This research project is developing the use of fecal aldosterone assays as an additional measure of adrenal activation during stress responses in North Atlantic right whales (*Eubalaena glacialis*), and is exploring the feasibility of measuring stress-related hormones in respiratory vapor from the same species.

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