

Assessing Stress Responses in Beaked and Sperm Whales in the Bahamas

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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 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) determine fecal hormone levels for relatively un-disturbed reference populations of these two deep-diving whale species off Great Abaco Island in the Bahamas, and 2) 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 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 FY2013 were to:

- (1) Conduct dedicated fecal sampling surveys (30 days) for reference populations of Blainville's beaked whales off southwest Great Abaco Island. Sperm whale samples were also collected opportunistically. Surveys were accompanied by concurrent photo-identification to identify individual whales and obtain associated life history data.

- (2) Refine field equipment and methods to maximize fecal sampling rates from beaked whales.
- (3) Set-up a field laboratory in the Bahamas for preliminary processing of samples shortly after collection.

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). This research project alternates annually between fieldwork by the BMMRO off Great Abaco Island in the Bahamas to collect fecal samples from relatively undisturbed populations of Blainville's beaked whales and sperm whales, followed by a year of laboratory analyses and data interpretation at the NEAq Marine Stress Laboratory. Fecal sample collection is being led by BMMRO scientists (D. Claridge, C. Dunn) with assistance from NEAq scientists (R. Rolland, S. Kraus). BMMRO is providing individual identification (based on photo-identification) and life history information on sampled whales. Endocrine analyses and data interpretation are being conducted by the NEAq (R. Rolland, K. Hunt, S. Kraus).

In the first year of the project (FY 2011), 30 days of fieldwork yielded 19 fecal samples from beaked (n=9) and sperm whales (n=10), demonstrating the feasibility of the field sampling approach (Rolland et al. 2011). In the second project year (FY 2012), we successfully validated radio-immunoassays for the fecal metabolites of three reproductive hormones (estrogens, progestins, androgens), adrenal glucocorticoids (GCs) and thyroid hormone (tri-iodothyronine, T3) in both species (Rolland et al. 2012) using adaptations of previously published methods (Hunt et al. 2006; Rolland et al. 2005; Wasser et al. 2012). Therefore, the first two years of the project established the validity and applicability of fecal hormone assay methodology to develop baseline levels for five fecal hormones, including two stress-related hormones (GCs and T3) in these two species. The following two option years include an additional 30 days of sample collection in the Bahamas (FY 2013) to increase sample sizes from whales of different life-stages (with a primary focus on beaked whales), and a final year (FY 2014) to assay the newly collected samples, complete data analyses and interpretation, and write the final project report.

This Annual Report details work completed during the first option year in FY 2013, which focused on sample collection in the Bahamas, refinement of sampling methods, and setting up laboratory equipment for initial sample processing in the Bahamas.

WORK COMPLETED

Task 1. Sample Collection in the Bahamas

Fieldwork was conducted by BMMRO aboard a 6.8m rigid-hulled inflatable boat between July – September 2013, with an emphasis on dedicated surveys to collect fecal samples from beaked whales off southwest Great Abaco Island. We prioritized beaked whales to maximize sample collection from the species of primary interest. Sperm whale samples were collected when whales were encountered opportunistically. Sample collection was accompanied by photographic documentation of all whales encountered, and photographs of individual sampled whales were later compared to existing identification catalogues (maintained by BMMRO) 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 whales defecate subsurface (~ 3-4 m) in the water column, and samples were collected by towed divers equipped with a small dip-net and a plastic- zipper bag using methods previously developed by BMMRO and NEAq (Rolland et al. 2011). To provide assistance for the fieldwork, NEAq hired an experienced assistant for the month of July, and BMMRO brought on additional assistants (interns and volunteers) for the entire field effort. During the first two weeks of July extensive training of field personnel in beaked whale sampling techniques was conducted with multiple practice sessions using subsurface collection of plumes of coffee grounds. Because sample weights from beaked whales were low in the first year of fieldwork (FY 2011), new sampling nets were designed to increase retention of fecal material. These custom-made nets (Florida Aquatic Nets), consist of a stainless steel hoop (7" x 7"), a 6" net of brine-shrimp fine nylon mesh netting, and vinyl rims with an attached lanyard (Figure 1). Using these nets appeared to increase collected sample mass significantly.



Figure 1. Custom-designed stainless steel hoop nets used to maximize fecal sample collection weights from beaked whales.

Because beaked whale samples are collected sub-surface, a volume of seawater is scooped into the plastic bags along with the fecal material in the nets. During sample processing and validation of the hormone analyses in FY 2012 (Rolland et al. 2012), we determined that some polar hormone metabolites had migrated into the seawater inside the sample jars from the beaked whale fecal material. To address this issue, in FY 2013 we set-up a field laboratory in the Bahamas (Figure 2). A used portable centrifuge (IEC CL2) was shipped to the Bahamas along with lab supplies, and bench space was constructed at BMMRO. The day of collection, fecal samples were spun (10min x 3000rpm), 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. Both sample types will be analysed for hormones at the NEAq laboratory in Option Year 2 of the project.

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 (Rolland et al. 2011).

Task 2b. Hormone Assays

Samples collected in FY 2011 were assayed in FY 2012, and the results have been analyzed and reported to ONR (Rolland et al. 2012). Samples collected in FY 2013 will be analyzed during Option Year 2 of the grant.

Task 3. Data Analysis, Publications and Reporting

Preliminary analysis of hormone results occurred in FY 2012, but the majority of analyses and publication writing will occur in Option Year 2 when all data are available. Results from the first two years of the project will be presented orally at the upcoming Biennial Meeting of the Marine Mammal Society (December 2013) in Dunedin, New Zealand.



Figure 2. The field laboratory set-up in the Bahamas with a portable centrifuge to remove excess water from fecal samples shortly after collection.

RESULTS

Field Effort

The survey team were on standby for a total of 66 days (between July 1- September 22, 2013), but due to especially poor weather conditions for the time of year, dedicated sampling surveys could only be conducted on 18 vessel-days (total of 14 full days). The success of beaked whale sampling, in particular, is dependent upon a Beaufort seastate ≤ 2 and good underwater visibility, and the Bahamas weather this summer featured numerous tropical storm systems with associated precipitation and high winds. Because BMMRO was unable to conduct surveys on the full 30 days that were budgeted, we are planning to request a no cost extension from ONR to allow us to use the remaining field time in FY 2014 for additional sample collection.

Surveys were primarily concentrated near the 1000 m isobaths along the southwest side of Great Abaco Island, in the northern Bahamas (Figure 3). Survey effort totaled over 500 km of vessel track lines.

There were 44 cetacean sightings during surveys, including eight different species. The research team located 12 groups of Blainville's beaked whales and 9 groups of sperm whales, and spent a total of 19.4 hours and 9.3 hours, respectively, following groups (Table 1). Group size ranged from 1-6 whales for Blainville's beaked whales (median = 3.5) and 1-3 animals for sperm whales (median = 2).

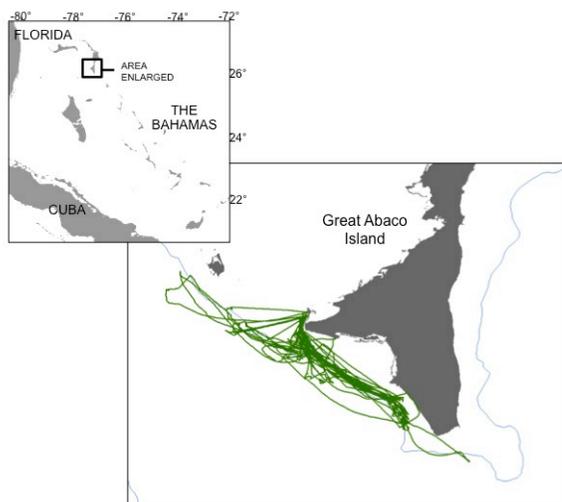


Figure 3. Map showing vessel tracks (green lines) during surveys off the coast of Great Abaco Island during July-September 2013. Survey efforts were concentrated off the southwestern side of the island along the 100 m isobaths (shown as blue line).

Table 1. Summary of field effort.

EFFORT DATA							
Sample # or Encounter (No Sample)	Date	Enc. Seq.	Begin Time Enc.	End Time Enc.	Length of Enc. (min)	Group size	Group Composition
Blainville's beaked whale encounters							
no sample	7/15/13	1	10:09	10:28	19	1	
Md 13-01	8/25/13	1	9:25	9:36	11	6	1AM, 2AF, 1SA 2 Calves
no sample	8/25/13	2	11:40	13:12	92	2	1SM 1SU OR 1AF 1JU
Md 13-02	8/27/13	1	10:20	10:42	22	5	2AF, 2calves, 1 AM
no sample	8/27/13	2	10:46	10:54	8	2	1SM 1SU
Md 13-03	9/3/13	1	15:16	16:04	48	2	2SA
no sample	9/7/13	1	15:59	16:22	23	2	1AM, 1SA / A F
Md 13-04	9/12/13	1	17:14	17:56	42	2	1AM, 1SM
Md 13-05	9/13/13	1	8:56	18:50	594	5	1AM, 1SA, 3AF
Md 13-06	9/13/13	1	8:56	18:50	594	5	1AM, 1SA, 3AF
Md 13-07	9/13/13	1	8:56	18:50	594	5	1AM, 1SA, 3AF
Md 13-08	9/13/13	1	8:56	18:50	594	5	1AM, 1SA, 3AF
Md 13-09	9/13/13	1	8:56	18:50	594	5	1AM, 1SA, 3AF
Sperm whale encounters							
Pm 13-01	8/25/13	3	13:58	14:05	7	1	1SM
Pm 13-02	9/09/13	3	14:58	15:19	21	3	2SM, 1 Unk
Pm 13-03	9/12/13	1	14:53	15:02	9	2	SM?, 1 Unk

Note: AM=adult male; AF=adult female; SM=subadult male; SU=subadult unknown sex; JU=juvenile unknown sex.

Fecal Sample Collection

Fecal samples were collected from throughout the survey area (Figure 4), but the majority of sampling took place off the southwest side of Great Abaco Island where survey effort was concentrated.

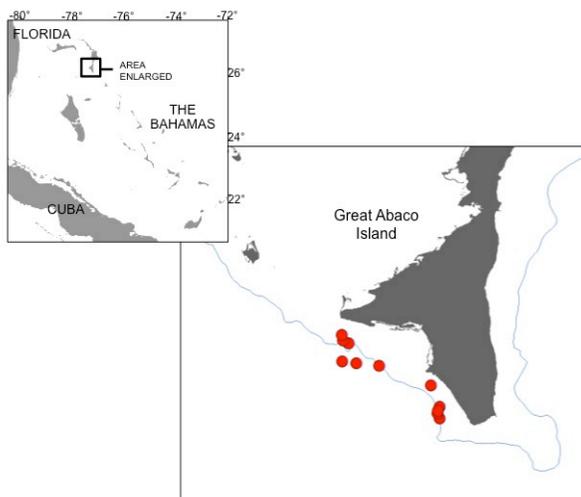


Figure 4. Map showing locations of Blainville's beaked whale sightings off Great Abaco Island during July-September 2013. The 1000 m line is shown.

Twelve fecal samples were successfully collected from Blainville's beaked whales ($n = 9$) and sperm whales ($n = 3$) during the field effort (Table 2). There were no repeat collections from the same individual. As many as five samples were collected from beaked whales in a single day, repeating the maximum samples collected in a day in FY 2011 (Table 1). In addition, BMMRO collected another 11 samples opportunistically from sperm whales during other activities not funded by this grant between 2011-2013 (data not shown). Therefore, a total of 23 additional fecal samples are available for hormone analyses.

Most of the beaked whale samples came from identified whales in the BMMRO Catalog, but three samples were from new whales. Two of three sperm whales sampled were also identified. Associated whale data including information on the age-class, sex, and reproductive status based on individual life histories for known whales are summarized in Table 2. We collected samples from all age-classes, including a female that appeared to be pregnant.

Table 2. Summary of individual life history data for known whales.

INDIVIDUAL LIFE HISTORY DATA					
Sample #	Whale ID	Sex*	Age class	Reproductive State	Notes
BLAINVILLE'S BEAKED WHALES					
Md 13-01	Unknown	Unknown	Calf	Immature	
Md 13-02	Unknown	Female	Adult	Possibly pregnant	
Md 13-03	Unknown	Unknown	Subadult	Immature	
Md 13-04	Md300	Male?	Subadult	Immature	first seen in 2011

Md 13-05	Md198?	Female?	Subadult	Immature	first seen in 2005
Md 13-06	Md094	Female	Adult	Mature	first seen in 1998
Md 13-07	Md190	Female	Adult	Mature	first seen in 2003
Md 13-08	Md134	Female	Adult	Mature	first seen in 1999
Md 13-09	Md305	Male	Adult	Mature	first seen in 2012
<i>SPERM WHALES</i>					
Pm 13-01	Pm156	Male?	Subadult?	Immature?	first seen in 2007
Pm 13-02	Unknown	Male?	Subadult?	Immature?	
Pm 13-03	Pm106	Male?	Subadult?	Immature?	first seen in 2002
*If ID is unknown, age and sex class information is from visual observations in the field					

Including both fieldwork years (FY2011 and FY 2013) and opportunistic samples, total fecal samples collected for this project to date is 42 (Table 3).

Table 3. Summary of all fecal samples collected to date from Blainville’s beaked whales and sperm whales off Great Abaco Island that are available for hormone analyses. “Other” refers to samples collected opportunistically by BMMRO (2011-2013).

Year	Beaked Whales	Sperm Whales	Total
2011	9	10	19
2013	9	3	12
Other	0	11	11
	18	24	42

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 (D’Amico et al. 2009). These events have involved Cuvier’s (*Ziphius cavirostris*) and also Blainville’s beaked whales (in smaller numbers), and include an atypical mass stranding in the northern Bahamas in mid-March of 2000 (Balcomb & Claridge 2001). In addition to these known mortalities, it is unknown whether periodic and 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).

Developing methods to better understand the 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 measurement of stress-related fecal hormones can be successfully applied in beaked whales and a co-occurring cetacean, sperm whales. This is a viable approach to characterize stress responses to underwater noise and other stressors in these two deep-diving whale species, with the advantage that samples are obtained non-invasively causing no additional disturbance to the research subjects.

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 exudates from the same species.

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