

## **Development of Respiratory Sampling to Assess Stress Responses in North Atlantic Right Whales**

Kathleen E. Hunt, Ph.D.  
Research Scientist  
New England Aquarium  
Central Wharf  
Boston MA 02110  
phone: (617) 226-2175 fax: (617) 973-0242 email: [huntk@neaq.org](mailto:huntk@neaq.org)

Rosalind M. Rolland, D.V.M.  
Senior Scientist  
New England Aquarium  
Central Wharf  
Boston, MA 02110  
phone: (617) 973-6587 fax: (617) 973-0242 email: [rrolland@neaq.org](mailto:rrolland@neaq.org)

Scott D. Kraus, Ph.D.  
Vice President of Research  
New England Aquarium  
Central Wharf  
Boston MA 02110  
phone: (617) 973-5457 fax: (617) 973-0242 email: [skraus@neaq.org](mailto:skraus@neaq.org)

Award Number: N000141110435

[http://www.neaq.org/conservation\\_and\\_research/projects/project\\_pages/right\\_whale\\_research.php](http://www.neaq.org/conservation_and_research/projects/project_pages/right_whale_research.php)  
[http://www.neaq.org/conservation\\_and\\_research/projects/conservation\\_medicine/index.php](http://www.neaq.org/conservation_and_research/projects/conservation_medicine/index.php)

### **LONG-TERM GOALS**

Our primary long-term goal is to assess whether respiratory sample ("blow") analysis can be developed as a practical method of physiological stress assessment in free-living cetaceans. Few methods exist for assessment of physiological stress levels of free-swimming cetaceans (Amaral 2010, ONR 2010). Respiratory samples (blow) can potentially be collected from targeted individuals and from large numbers of whales. Additionally, blow sampling is non-invasive and should not alter the stress response being measured, and it can be employed multiple times on known individual whales. Overall, blow may offer a novel method for assessment of short-term stress (minutes/hours) in cetaceans, and may offer a useful complement to existing fecal-sampling methods (Rolland et al. 2005, Hunt et al. 2006).

### **OBJECTIVES**

Our primary objective in FY2011 was to test and develop a practical methodology for collecting respiratory samples from free-swimming, large baleen whales. Our second objective (FY2012) will be

# Report Documentation Page

Form Approved  
OMB No. 0704-0188

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE <b>30 SEP 2011</b>		2. REPORT TYPE		3. DATES COVERED <b>00-00-2011 to 00-00-2011</b>	
4. TITLE AND SUBTITLE <b>Development of Respiratory Sampling to Assess Stress Responses in North Atlantic Right Whales</b>				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>New England Aquarium, Central Wharf, Boston, MA, 02110</b>				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release; distribution unlimited</b>					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>			

to test these respiratory samples for hormone content, focusing primarily on stress-associated hormones such as glucocorticoid and thyroid hormones, and secondarily on reproductive hormones (estrogens, progestins, androgens).

## **APPROACH**

Our approach in FY2011 involved:

- (1) Design and construction of a carbon-fiber pole and associated boat mount that can be attached to a small research vessel, modifying the designs used by Hogg et al. (2005), Hogg et al. (2009), and Acevedo-Whitehouse et al. (2010), with the boat mount modified from designs by Woods Hole Oceanographic Institution (WHOI).
- (2) Design, testing and construction of sample collectors that can be mounted interchangeably on the end of the pole, with the aim of designing cost-effective collectors that can be readily constructed from easily available materials, and that collect and retain droplets of blow vapor. Our main approach involves modification of the nylon-fabric collector used by Hogg et al. (2009), e.g. a plastic framework holding a mass of absorbent fabric, but redesigned to collect larger sample volumes. Additionally we tested modifications of this design, including other types of absorbent and also plate-type collectors.
- (3) Field testing of the pole and collectors with a well-studied population of North Atlantic right whales (*Eubalaena glacialis*; NARW) in the Bay of Fundy during the months of August and September, 2011. Our specific goal for fieldwork for FY2011 was to collect 35 samples from free-swimming North Atlantic right whales during 7 days of boat work in the Bay of Fundy.
- (4) Initial laboratory testing to determine whether respiratory samples contain a mucoid fraction that can be freeze-dried or centrifuged. (The remainder of laboratory testing will occur in FY2012).

Key individuals in this project are: PI Kathleen Hunt, Ph.D. (fieldwork, all laboratory analyses and general oversight); Co-PI Rosalind Rolland, D.V.M. (pole operation, health assessment and data analysis); Co-PI Scott Kraus, PhD. (pole design/construction, boat piloting and other field logistics).

## **WORK COMPLETED**

### ***Task 1: Testing Field Collection Techniques for Respiratory Sampling***

In FY2011 we designed and constructed the carbon-fiber pole and boat mount, and attached the boat mount to the bow of our 7.6m research vessel *Callisto*. We developed a method to attach a variety of different collectors to the end of the pole (see below). We extensively tested this system with free-swimming NARW in the Bay of Fundy during August-September 2011.

We also developed a collector apparatus that regularly yields samples of >50uL volume. After initial trials on land on over a dozen possible designs, we tested the following four blow-collector designs on water: (1) plastic bottles wrapped with an external layer of nylon stocking (based on previous collection methodology used by Hogg et al. 2009), (2) plastic bottles containing an internal wad of nylon tulle fabric, (3) plastic bottles wrapped with an external layer of nylon tulle fabric, (4) a flat plexiglass plate with droplets collected by "squeegee" after passing the plate through the blow cloud. Multiple sub-types of these collector apparatuses were also tested (e.g. short vs. tall bottle types, single vs. double vs. multiple layers of fabric).

### ***Task 2a: Laboratory Validations***

Only two preliminary laboratory tests were scheduled for FY2011. The first involved developing a method to centrifuge the absorbent material of the blow collectors, and thus verifying that we were obtaining enough sample volume for subsequent assay testing. We performed these tests in early September 2011, developing a custom-built apparatus for centrifugation of large masses of absorbent material, and verified that we are collecting sufficient sample volume. Our second laboratory goal was to test whether centrifugation of samples produces a visible pellet or mucoid layer, and hence whether freeze-drying or centrifugation can be used to concentrate samples. We performed this test in early September 2011.

### ***Task 2c: Assaying Individual Blow Samples for Stress and Reproductive Hormones***

### ***Task 3: Data Analysis and Reporting***

Both these tasks will occur in FY2012.

## **RESULTS**

We successfully developed and tested a practical method of blow collection from free-swimming large baleen whales. Our final method involves a 32' extendable carbon-fiber pole that is suspended on a swiveling mount attached to the bow of a small vessel. The pole can be maneuvered over the blowholes of a whale by a pole operator standing at the bow of the boat (Figures 1, 2). A clamp on the end of the pole allows attachment of a variety of different devices. The collector type producing greatest sample volume was a plastic bottle wrapped with an outer layer of absorbent material (Figure 3). Plate-type collectors also performed well in initial testing. Our collection method requires a minimum crew size of four (boat pilot, pole operator, sample-processor/data recorder, and photographer; Figure 1).



*Figure 1. Research vessel Callisto with 32' blow-collecting pole partly extended over a North Atlantic right whale. Photo: Moira W. Brown.*



*Figure 2. Research vessel Callisto with 32' blow-collecting pole fully extended over a North Atlantic right whale. Photo: Chris Slay.*



*Figure 3. Close-up view of a blow collector in position over the blowholes of a North Atlantic right whale. Photo: Amy R. Knowles.*

**Sample collection rate:** Despite anomalously poor weather conditions in the 2011 field season, we successfully collected 55 samples in 7 partial boat days, greatly exceeding our goal of 35 samples in 7 full boat days. We had anticipated a sample collection rate of approximately 5 samples per full boat day, yet our overall average was 7.9 samples per day, and this rate includes the first several days when we were still practicing with the pole, and also includes partial days with poor weather conditions (sea state 4 or higher for part of the day). In our last three days of boat work, i.e. once we had attained good skill with the pole, our average sample collection rate jumped to 14.7 samples per day. Our highest collection rate (23 samples/day) occurred when working with whales in a large courtship group (or SAG, surface active group). When following individual whales that were not in SAGs, our collection rate was typically 10 samples/day. On a per-hour basis, if whales were present and weather conditions were good (sea state of 3 or less), we collected an average of 2.2 samples per boat hour (not including transit time). Deployment of the collection apparatus is rapid enough to re-sample an individual whale during a single surfacing sequence. Nearly 100% of our samples were from photographed whales that will be linked to identified whales in the North Atlantic Right Whale Catalog after photo analysis, providing life history data and visual health assessment information (Pettis et al. 2004, 2006). Based upon preliminary field identifications, blow samples were collected from both sexes and a variety of reproductive states.

**Sample volume and type:** The majority of samples are between 10-500uL in volume, with a few samples over 1000uL. In many cases additional sample is still retained in the absorbent material (currently in storage in -80C freezers) and will likely be recovered later upon further processing in the lab. Samples appear to be primarily aqueous, and centrifugation produces no visible pellet and no visible mucoid layer. Thus, forthcoming laboratory analyses in FY2012 will use "whole" blow (uncentrifuged and not freeze-dried).

## **IMPACT/APPLICATIONS**

The procedure that we have developed represents an effective practical technique for collection of respiratory vapor from large baleen whales. We expect that other marine mammal researchers may be able to use this collection method, not only for endocrine analysis but also for a variety of other research purposes (e.g., studies of respiratory bacteria, DNA sampling, etc.). The work described here may add to the tools needed to evaluate the physiologic consequences of different noise levels on one species of baleen whale, and may serve as a model for other marine species.

## **RELATED PROJECTS**

No related projects.

## **REFERENCES**

- Acevedo-Whitehouse K., Rocha-Gosselin A., and Gendron D. 2010. A novel non-invasive tool for disease surveillance of free-ranging whales and its relevance to conservation programs. *Animal Conservation* 13:217-225.
- Amaral, R.S. 2010. Use of alternative matrices to monitor steroid hormones in aquatic mammals: a review. *Aquatic Mammals* 36:162-171.
- Hogg, C.J., Vickers, E.R., and Rogers, T.L. 2005. Determination of testosterone in saliva and blow of bottlenose dolphins (*Tursiops truncatus*) using liquid chromatography - mass spectroscopy. *Journal of Chromatography B*:814(2):339-346.
- Hogg, C.J., Rogers, T.L., Shorter, A., Barton, K., Miller, P.J.O., and Nowacek, D. 2009. Determination of steroid hormones in whale blow: It is possible. *Marine Mammal Science* 25(3):605-618.
- Hunt K.E., Rolland R.M., Kraus S.D., and Wasser S.K. 2006. Analysis of fecal glucocorticoids in the North Atlantic right whale (*Eubalaena glacialis*) using high-performance liquid chromatography (HPLC). *General and Comparative Endocrinology* 148:260-272
- Office of Naval Research. 2010. Final Workshop Proceedings, "Effects of Stress of Marine Mammals Exposed To Sound", Arlington, VA; meeting dates 4-5 November 2009.
- Pettis, H.M., Rolland, R.M., Hamilton, P.K., Brault, S., Knowlton, A.R., and Kraus, S.D. 2004. Visual health assessment of North Atlantic right whales (*Eubalaena glacialis*) using photographs. *Canadian Journal of Zoology* 82(1):8-19.
- Pettis, H.M., Rolland, R.M., Hamilton, P.K., Knowlton, A.R. and Kraus, S.D. 2006. Visual assessment of North Atlantic right whale (*Eubalaena glacialis*) health and its relationship to reproduction and survival. Final Report to the National Marine Fisheries Service. Contract # NAO3NMF4720464.

Rolland, R.M., Hunt, K.E., Kraus, S.D. and Wasser, S.K. 2005. Assessing reproductive status of right whales (*Eubalaena glacialis*) using fecal hormone metabolites. *General and Comparative Endocrinology* 142:308-317.