

December
2014

Vessel Survey Plan

for
**Mid-Atlantic Humpback Whale
Monitoring,
Virginia Beach, VA**

In Support of
**Contract Number N62470-10-3011
Task Order 54**



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Acronyms and Abbreviations

AW	Allied Whale
CCS	Center for Coastal Studies
COA	College of the Atlantic
COR	Contracting Officer's Representative
GPS	Global Positioning System
MINEX	Mine Neutralization Exercise
MMO	Marine Mammal Observer
NARW	North Atlantic Right Whale
NAVFAC LANT	Naval Facilities Engineering Command Atlantic
NOAA	National Oceanic and Atmospheric Administration
USFF	United States Fleet Forces
VAQF	Virginia Aquarium & Marine Science Center Foundation

Section 1 Survey Summary

Cruise Title: Mid-Atlantic Humpback Whale Monitoring.

Survey Dates: Surveys will commence in December of 2014 and extend through the spring of 2015. Twenty (20) nearshore survey days are planned with an additional five (5) offshore survey days as weather allows.

Study Area: The nearshore study area will encompass waters in and around the mouth of the Chesapeake Bay as well as the W-50A Mine Neutralization Exercise (MINEX) region off Virginia Beach, VA (**Figure 1**). The offshore study site will encompass an approximately 3,138 km² area, extending to the continental shelf and including Norfolk Canyon (**Figure 2**).

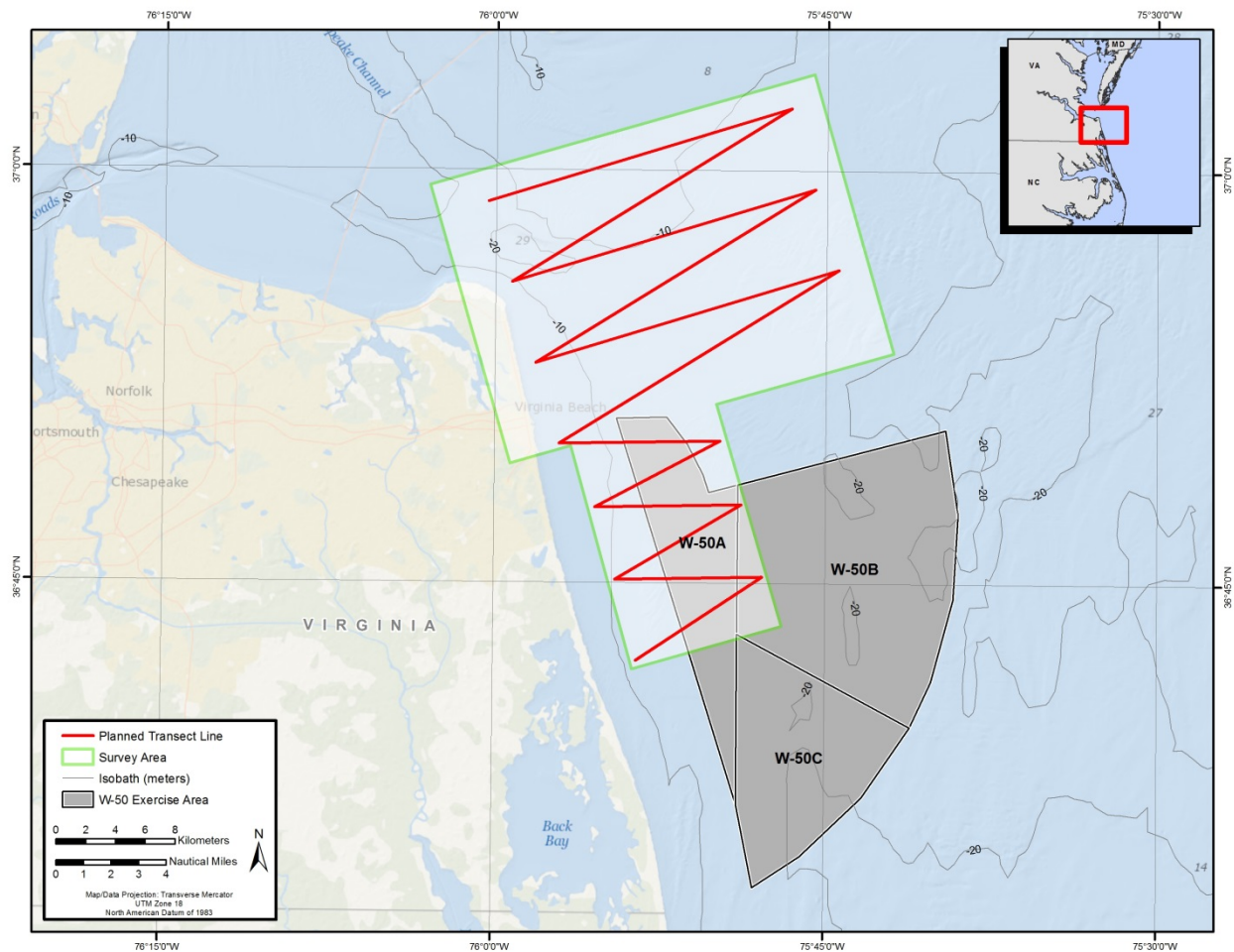


Figure 1. Map of the nearshore study area, which includes waters in and around the mouth of the Chesapeake Bay as well as the W-50A MINEX region off Virginia Beach. Red lines indicate transect lines that will be run when there are no reports of humpback whales in the area.

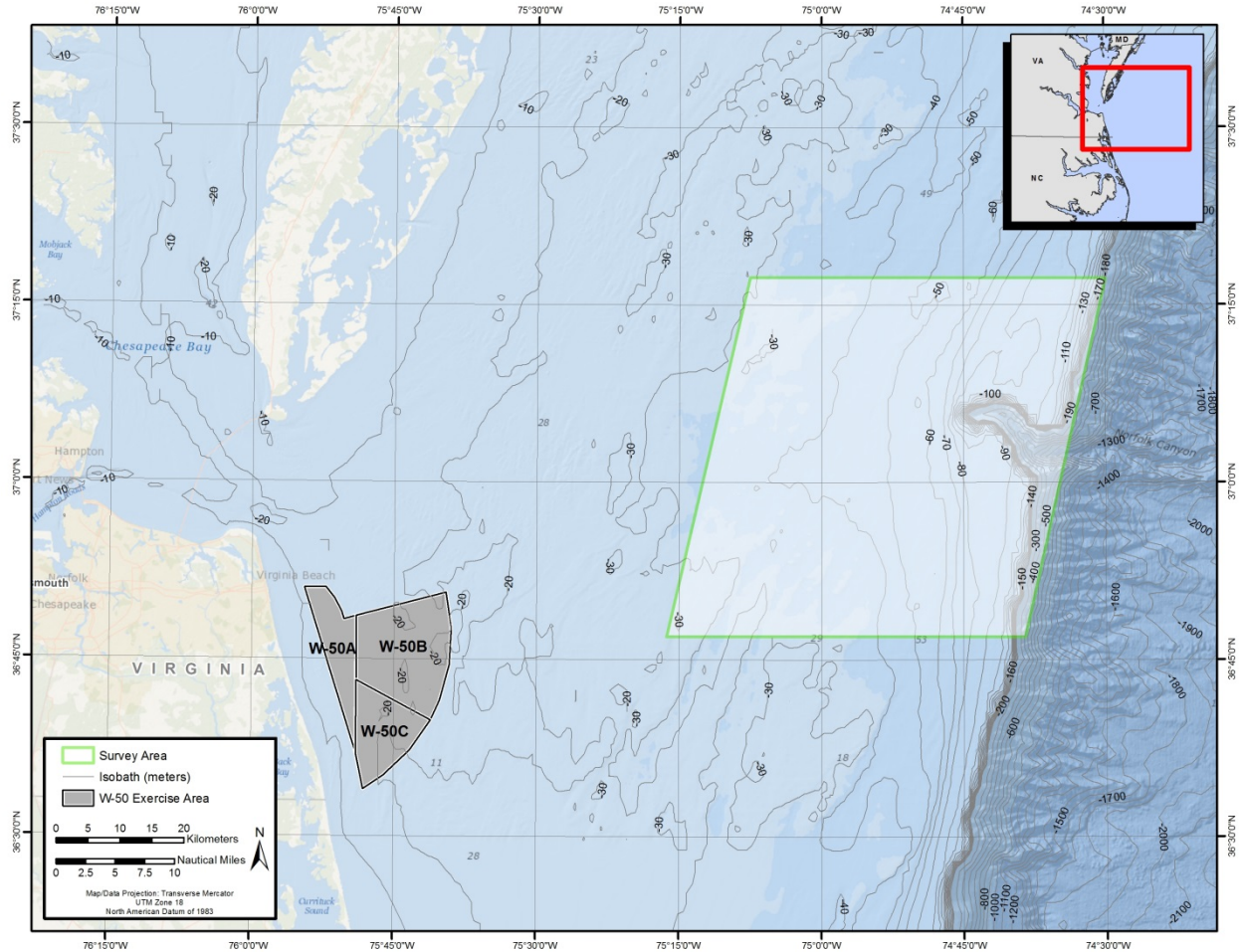


Figure 2. Map of the offshore study area that extends out to the continental shelf and includes Norfolk Canyon. Survey efforts will be focused in this region during the 5 days of offshore surveys.

Research Vessel: 23' Willard Marine 730 LE R/V *Whale Research* (nearshore); 42' charter fishing vessel M/V *Flat Line* or other comparable vessel (offshore)

Sponsoring Institution: U.S. Navy – Naval Facilities Engineering Command Atlantic (NAVFAC LANT) and United States Fleet Forces (USFF) Command

Objectives: The objectives of this pilot project under the Navy’s Marine Species Monitoring Program are to establish baseline occurrence and behavior data for humpback whales in the Hampton Roads mid-Atlantic region by addressing the following questions:

- *What age classes (juveniles, sub-adults, adults) are utilizing the waters within and adjacent to the mouth of the Chesapeake Bay?*
- *Do humpback whales exhibit site-fidelity over periods of days to years?*
- *Do humpback whales congregate in specific high-traffic and/or*

high-use Navy training areas?

- *Do humpback whales spend significant time within or move through areas of U.S. Navy live-fire and mine neutralization exercises (Year 2)?*
- *What are the relative sound levels humpback whales are exposed to from vessel traffic and/or Navy training exercises (Year 3)?*

The project will consist of twenty days of nearshore surveys and five days of offshore surveys performing non-random, non-systematic surveys with the following objectives:

1. Obtain identification photographs of humpback whales (and other high priority species of baleen whales, e.g. North Atlantic right whales [NARWs]) for inclusion in regional and local catalogs.
2. Conduct focal follows of humpback whales (and other high priority species of baleen whales, e.g. NARWs) with an emphasis on priority Navy training areas, such as the W-50 MINEX zone, and shipping channels.
3. Collect biopsy samples of humpback whales for sex determination and mitochondrial control region sequencing as well as microsatellite genotyping of the tissue samples by University of Groningen and stable isotope analysis by Duke University.

Although the priority study area will be in Navy corridors, the PI may choose to expand the search for high priority species into other areas based on range closures, weather conditions, sighting reports, or other local information.

Key Personnel:

- ✓ HDR: Dan Engelhaupt (Chief Scientist), Amy Engelhaupt (Cruise Leader), Jessica Aschettino, and Dana Spontak.
- ✓ Navy: Joel Bell (Project Manager, NAVFAC LANT), Cara Hotchkin (NAVFAC LANT), Jackie Bort (USFF).
- ✓ Additional: Todd Pusser.

See **Appendix 1** for roles of each person.

Section 2 Survey Data Collection and Preparation

Vessel Operations

The nearshore survey vessel will be a 23' fiberglass hybrid foam collar boat, and all vessel operation decisions will be carried out by the Captain and Chief Scientist such that the safety of all personnel is the highest priority. For offshore surveys, a charter vessel will be used, most likely the 42' fishing vessel, Flat Line, which has been used on previous marine species surveys in the area and is fully licensed and insured for operation in offshore waters.

Twenty-five survey days will be conducted for this study, which includes twenty nearshore survey days and five offshore survey days. Vessel operations for nearshore survey days will depart from Marina Shores Marina, off Lynnhaven Inlet. Offshore survey days will depart from Rudee Inlet. Efforts will be coordinated with the W-50 MINEX range so that as often as possible the vessel will have clearance on the range. However, due to limited weather windows and the frequent lack of range clearance, it will not always be possible to conduct surveys when the range is open. During offshore survey days, an aerial team will fly in coordination with the vessel team to facilitate sighting and localization of humpback whales or other high priority species (e.g. North Atlantic right whales)

Survey Operations

The scientific crew will consist of 3-5 marine mammal scientists, which includes the boat driver on the nearshore surveys. The scientific crew will consist of 3-5 marine mammal scientists, not including the charter boat driver on the offshore surveys.

The surveys will be planned and scheduled for twenty-five full survey days. In order to maximize achieving the cruise objectives, surveys will commence as close as is practical to sunrise. If sea states reach a Beaufort 4, or visibility is reduced to less than one nautical mile due to rain or fog, the survey vessel may call off effort. Every effort will be made to avoid such circumstances by following the weather conditions closely before commencing a survey day.

Priority will be given to reports of whales in the survey area whenever available and the R/V will survey areas where known sightings have occurred. When no recent reports are available, the research team will transit to the study area and follow the pre-determined transects to survey for whales until a report comes through. During offshore survey days, the vessel will primarily coordinate with aerial surveys to increase chances of encounters rather than arbitrary tracklines.

Opportunistic observations of marine species will be conducted using a primary observation team of three individuals—two dedicated observers searching either naked eye or with 7 × 50 hand-held reticle binoculars or 10 x 30 hand-held image-stabilizing binoculars and the third observer (boat driver) with unaided eyes. Once a sighting commences, one observer will focus on data recording while the other focuses on obtaining photo identification images of the individual(s).

The data recorder's primary duties will be to record information into the iPad Focal Follow app, which also automatically records a time-stamped position of the vessel every 30 seconds. When

not entering data, the recorder will visually scan a 180-degree sweep in front of the vessel with naked eye and use the binoculars to more closely search for any cues.

The vessel will maintain a survey speed of approximately 10-12 knots during search efforts. When an aerial team is working in conjunction with the boat team, the Captain will maintain contact with the aerial team.

During a marine mammal sighting, or when in the vicinity of a recently reported sighting, the research vessel will attempt to approach the animal(s). The approach will be done in a manner to minimize disturbance to the animals and to maximize the scientific crew's abilities to confirm species, obtain group size estimates, and obtain photo-identifications and high definition video. Focal follows will be attempted on all groups of baleen whales. Less time will be spent collecting information on lower priority species, and more time on higher priority species. The decision on when to end data collection efforts on a particular sighting or to switch to a different sighting will be made by the Cruise Leader.

Areas of Operations

The operating area for each day will be chosen depending on weather conditions and reports of humpback whales or other baleen whales in the area. There will always remain a high focus on areas of high Navy use, such as the W-50a MINEX zone (see **Figure 1**) and shipping channels. If there are no reports of humpback whales or other baleen whales in the primary survey area, the research vessel will follow a pre-determined set of tracks that cover the high priority regions, although these will not follow line-transect survey methodology.

Photography

Every effort will be made to obtain good quality identification photos of the flukes and/or dorsal fins of high priority marine mammal species encountered. Humpback whales occurring in shallow waters often do not present their tail flukes while diving, so while tail fluke images are ideal to collect, dorsal fin images are likely to be more obtainable. At the end of each survey day, photos will be cropped and compiled in a format suitable for data sharing with other North Atlantic humpback whale catalogs. HDR will share their images with known regional catalogs, including those curated by the Provincetown Center for Coastal Studies (PCCS) and Allied Whale (AW)/College of the Atlantic (COA), as well as local catalogs, such as the catalog maintained by Virginia Aquarium Foundation (VAQF) in an effort to further the science on these endangered species.

Logging of Data, including Focal Follows

During the survey, the data recorder will maintain a log of observers, environmental sighting conditions, and sighting information in the iPad Focal Follow program. Environmental data will be updated when sighting conditions change. When a sighting is made, information regarding the distance and bearing to the sighting, species identification, speed and direction of the animals, group size, photo, and video information will be logged when available. See **Appendix 2** for a list of data to be recorded.

Sighting distances will be calculated using reticles or by estimation if no horizon is visible or initial sighting is made by naked eye. Location data and vessel speed will be obtained from a

global positioning system (GPS) unit feeding directly into the iPad Focal Follow survey software.

Biopsy Sample Collection

After the survey team has finished completing focal follows of the target species and photo-identification photographs have been obtained, biopsy samples may be collected. Every effort will be made not to re-sample individuals sampled previously by the VAQF by comparing photographic images of known biopsied individuals with those of the possible target individual. Photographs of previously biopsied individuals will be stored on an iPad so that they may be compared in the field. Biopsy samples may be collected with a Paxarm gun or with a Barnett crossbow. Skin samples will be wrapped in aluminum foil and placed in a Whirlpak bag after collection and stored in an ice cooler on the vessel. At the end of the day, samples will be cross-sectioned, placed in the appropriate cryovial, and stored in a freezer until they are ready for shipment. See **Appendix 3** and **Appendix 4** for detailed information on the processing of samples for Duke University and University of Groningen.

Chief Scientist/Cruise Leader

The Chief Scientists/Cruise Leaders are Dr. Dan Engelhaupt and Amy Engelhaupt, HDR Inc. The Cruise Leaders are authorized to modify these cruise instructions provided that the proposed changes will not:

- Jeopardize the safety of personnel or the ship
- Exceed the time allotted for the cruise
- Result in undue additional expense
- Change the general intent of the project.

The Cruise Leaders will notify the Navy Technical Contracting Officer's Representative (COR) at NAVFAC LANT and the Survey Coordinator of any significant deviations to the cruise plan.

Participating Scientists

Please refer to **Appendix 1**. All personnel will have a U.S. government-issued identification onboard.

Scientific Collection Permit

All data collected will follow the specification and limitations described in NOAA Scientific Research Permit number 16239 issued to Dan Engelhaupt, HDR and/or NOAA Scientific Research Permit number 14450 issued to Dr. Keith Mullin, NMFS where Dan Engelhaupt is listed as a Co-PI. All biopsy samples will be collected under permit #14450. All members of the scientific crew will be asked to review the permit in accordance with its terms.

Equipment

Please refer to **Appendix 5** to see the equipment list.

Data Responsibilities

Data will be compiled following the survey and back ups will be made of all data and photographs collected from each survey day. All data, photographs, and video collected from the survey vessel is considered property of the U.S. Navy and will not be distributed or used for any purpose not specifically authorized by the COR. Sighting data will be uploaded to the Ocean Biogeographic Information System Spatial Ecological Analysis of Megavertebrate Populations (OBIS-SEAMAP) database, managed by Duke University, and photo-identification catalog images will be shared with AW/COA, PCCS, and/or VAQF as appropriate.

Communications

The scientific crew will have a minimum of two hand-held radios. The Cruise Leader will have a cellular and hand-held satellite phone as well as an EPIRB. The scientific crew will coordinate any communications with the Cruise Leader. Emergency telephone calls can be made to 011-8816-2245-2433. The Cruise Leader will also maintain daily communication with the designated shore contact to update ship position(s) and planned areas of operation. The shore contact will be determined on a day-to-day basis based on availability.

Reports

HDR will provide a written annual progress summary report and presentation at the Navy's annual Monitoring Program Technical Review Meeting, as well as a final technical project report at the conclusion of the project. The report will include an abstract, introduction, objectives, methods section, results, and a discussion with conclusions. All data collected on the survey will be available for inclusion in the report. These may include, but are not limited to, photographs, videos, GPS tracklines, sighting information (in a Microsoft Excel format, and any other information collected or generated during the survey including raw data, GPS data, and GIS files). The results of this study and associated analyses are expected to be presented in peer-reviewed publications.

Pre-Cruise Preparation

Prior to the first survey, the Cruise Leader will provide the scientific team with a copy of these cruise instructions, and reiterate the survey objectives, procedures, ship rules, emergency response plan, and the HDR Health and Safety Plan. The scientific crew will also receive an overview on using the iPad app for collecting focal follow data.

Safety of the Crew and Vessel

Safety of the crew will always be the priority. If the safety of the crew or the vessel is jeopardized at any time, survey operations will be suspended immediately.

The Cruise Leader/Chief Scientist serves as the Site Safety and Health Officer and will make on-the-spot decisions concerning safety and has the authority to terminate the survey as necessary to ensure safety of the crew and team. As noted earlier, the scientific crew will be briefed on health and safety procedures, including emergency contact procedures. All survey operations will be suspended in the event of a medical emergency.

See **Appendix 6** for an Emergency Contact list.

Appendix 1 – Scientific Personnel for the Survey

Crew Position	Name	Company
Captain/Cruise Leader	Dan Engelhaupt	HDR
Captain/Cruise Leader/ Marine Species Observer	Amy Engelhaupt	HDR
Cruise Leader/Marine Species Observer	Jessica Aschettino	HDR
Marine Species Observer	Dana Spontak	HDR
Marine Species Observer	Todd Pusser	Independent Contractor
Marine Species Observer/Navy Project Manager/Technical COR	Joel Bell	NAVFAC LANT
Marine Species Observer	Cara Hotchkin	NAVFAC LANT
Marine Species Observer	Jackie Bort	USFF

Appendix 2 – Data fields to be recorded in iPad App

Placement	Field / Attribute
Survey/ Environmental	Date / Time Platform Survey ID Beaufort Scale Visibility Wind Direction Swell Percent Cloud Cover Effort Status Leg Notes
Focal/Sighting (Related to Focal Individual Only)	Date / Time Latitude / Longitude Group ID Behavioral State (Travel; Feed; Mill; Social; Rest; Log; Unknown) Behavioral Event (Blow; Dive/Peduncle arch; FUD; FDD; Side fluke; Lunge; Tail slap; Pec slap; Spy hop; Breach; Bubbles; Start follow; Stop follow; Footprint WP; First surfacing; Head slap; Peduncle slap; Chase; Brood side display; Head lunge; Linear bubble trail; charge) Bearing Distance to Sighting Heading of the Animal Relative Movement of Vessel and Animal's Bearing Sighting Notes
Focal/Sighting (Related to Focal Individual and/or Group)	Photos (Y/N) Video (Y/N) Biopsy (Y/N) Tagging (Y/N) Maximum Distance between Nearest Neighbor Minimum Distance between Nearest Neighbor Depth
Focal/Sighting (Related to Group/Sighting)	Sighting Number Species Name (Common) Species Name (Scientific) Count (Best) Count (Calves) Recorder Observer Reaction Animal Speed Animal Bearing Initial Species ID Confirmed Species ID Min Group Size Max Group Size Best Group Size Min Calves Present Photos Taken (Y/N)Photos (Y/N) Video (Y/N) Biopsy (Y/N) Tagging (Y/N) Maximum Distance between Nearest Neighbor Minimum Distance between Nearest Neighbor Depth

* Upon each entry and time stamp and GPS coordinate is recorded for the position of the vessel. Variables may be modified as deemed necessary by the Cruise Leader.

Appendix 3 – Stable Isotope Analysis (Duke University)

WORK PLAN – STABLE ISOTOPE ANALYSIS FOR HUMPBACK WHALE BIOPSY SAMPLES

Danielle Waples (Research Analyst) will prepare the samples for stable isotope analysis, following the work flow document below. Sample preparation involves several laborious steps and will take multiple weeks to complete (For example, lipid extraction of samples is a four day process and homogenizing and packaging samples can take up to several weeks). The prepared samples will be delivered to the Duke Environmental Stable Isotope Laboratory for stable isotope analysis. Once analysis has been completed, Danielle will prepare a short report summarizing the isotopic results of the humpback samples.

Stable Isotope Work Flow

- 1) Remove any blubber attached to skin samples following clean protocols
 - a. If samples are frozen, minimize thawing time (process within 1-2 hours) to reduce the potential of bacterial transformation of carbon.
 - b. If sample are preserved in liquid, blot dry with a kimwipe or other lint-free cloth.
 - c. Clean scalpel in distilled water or methanol and wipe blade carefully with a kimwipe or other lint-free wipe, ensure no particles are transferred between samples.
- 2) Transfer skin samples to pre-labelled test tube and cover tube tops with duraseal film. Label test tubes with the sample's unique identifier.
- 3) Freeze dry samples. Prepare unit 24 hours in advance. Include one tube with water (when this is completely evaporated freeze drying is complete). Place all tubes in glass container and freeze to -80 before freeze drying. Freeze-drying can take up to 48 hours; check unit periodically. Once freeze-drying is complete, cover the top of each tube with an additional piece for duraseal film to keep samples from re-hydrating.
- 4) Lipid extract samples. Put each sample in a clean 15 ml glass vial. Pipette in 10ml of 2:1 chloroform/methanol. Shake and store in refrigerator overnight- cover with duraseal film. Remove solvent the next day and pipette in 10ml of new solvent then cover, shake and refrigerate. Repeat again on Day 3. After removing last round of solvent transfer samples to clean glass vial and let evaporate in hood. Take a subsample of all samples to UNCW to put in nitrogen evaporator to ensure all lipids have been extracted. Once this is confirmed, rinse all samples with distilled water and dry overnight at 50°C.
- 5) Homogenize the samples. Samples must be reduced to small uniform pieces using either a scalpel or ball mill. Transfer between 0.5 to 1.5 mg of homogenate to a tared 5x9mm silver capsule with a small spatula. Once the appropriate amount has been weighed, seal capsule by crimping the top of the capsule down. Gently squeeze down any sharp edges into a smooth ball or edgeless cube. Capsules puncture easily so this is a painstaking and time-consuming process.
- 6) Record the final weight of each capsule and sample. Place samples in a 96-well tray noting the unique ID of the sample's position in each tray. Transport samples to Duke Environmental Stable Isotope Laboratory in Durham. Store remaining samples in vials in a polypropylene box.

Appendix 4 – Treatment of Biopsy Samples in the Field (University of Groningen)

Treatment of biopsy samples in the field:

This protocol is solely directed towards using the sample for DNA analyses and pollutant studies. Ideally, the whole sample should be frozen in liquid nitrogen and kept at -80°C or lower in the laboratory. Such ideal treatment, however, is often infeasible in the field. An alternative is to preserve the sample in a storage buffer that works at ambient temperatures. The reason for wrapping the blubber part in aluminum foil is due to the fact that plastic contains many of the organochlorides, which are the target compounds for the pollution analysis.

Protocol:

- Remove the sample from the tip with cleaned (e.g., flaming or in 10% bleach and rinse) forceps and place it on a little piece of (acetone cleaned) aluminum foil.
- Separate the blubber from the skin using a metal scalpel with a clean blade.
- The blubber is wrapped in the aluminum foil and can be used for pollutant analyses apart from heavy metal analyses. Freeze the sample as soon as possible.
- The skin is put into a vial with 'pickle juice' (Amos and Hoelzel 1991).
- We use 3.6 ml Nunc Cryotubes™ which are made of polypropylene and has a silicone o-ring. Contrary to other brands e.g. Nalgene™, these vials do not leak even at the low pressures in the cargo room of an airplane.
- It takes time for the pickle juice to work. Accordingly the ideal is that sample vials are kept cool (5 degrees Celsius or frozen) at least for a week or so before subjected to ambient temperatures. Long-term storage as cold as possible.

NOTE: The pickle juice is comprised of DMSO and sodium chloride. You can smell the DMSO and while not directly unhealthy (people used to drink the stuff) we suggest that you work with open vials in a ventilated area.

References:

Amos W, Hoelzel AR (1991) Long-term preservation of whale skin for DNA analysis. *Report of the International Whaling Commission, Special Issue*, 13, 99–103.

Appendix 5 – Equipment List

Equipment
Survey Equipment
Barnett Wildcat crossbow
Paxarm biopsy gun
Paxarm biopsy supplies
Biopsy bolts and tips
Biopsy processing supplies
3 pairs 7x50 reticled binoculars
2 pairs Canon 10x30 IS binoculars
iPad with focal follow software
2 WAAS enabled GPS with BlueChart Virginia maps, charging cable, batteries
2 GoPro Hero4 cameras and accessories
1 X Canon 7D Mark II and 1 x Canon 7D with 100-400 mm telephoto lenses with cards, batteries, lens cleaner, pelican case
2 Hand-held VHF Radios
Other
NMFS Research Permits
Satellite Phone
EPIRB
Coast Guard certified lifejackets for all of crew on board

Appendix 6 – Emergency Contact List

Department	Telephone Numbers
United States Coast Guard:	Chesapeake, VA Main Phone: 757-421-6240 Emergency phone: 757-398-6321 Marine Radio – VHF Channel #16
Marine Forecast—Virginia Beach:	http://forecast.weather.gov/MapClick.php?site=akq&zmx=1&zmy=1&map_x=254.48187255859375&map_y=172.6455078125&x=280&y=190 NOAA weather marine VHF: channel 21
24-Hr Emergency Department:	911
Air Ambulance Service-Hampton Roads:	1-800-572-4354
HDR Satellite Phone:	8816 2245 2433
Hospital:	Sentara Virginia Beach General Hospital 1060 First Colonial Road Virginia Beach, VA 23454 Main phone: (757) 395-8000 Emergency phone: (757) 395-8890
HDR Program Manager/Chief Scientist:	Dan Engelhaupt (757) 354-6735 Dan.Engelhaupt@hdrinc.com
Point of Contact/Float Plan Contact:	Dana Spontak (830) 330-1834 Dana.Spontak@hdrinc.com

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