NOAA Technical Memorandum NMFS-SEFSC-670

doi:10.7289/V5CC0XN0

REMOTE BIOPSY SAMPLING FIELD PROCEDURES FOR CETACEANS USED DURING THE NATURAL RESOURCE DAMAGE ASSESSMENT OF THE MSC252 *DEEPWATER HORIZON* OIL SPILL

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February 2015

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February 2015

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This report should be cited as follows:

Sinclair, C., J. Sinclair, E. S. Zolman, A. Martinez, B. Balmer and K. P. Barry. 2015. Remote biopsy field sampling procedures for cetaceans used during the Natural Resource Damage Assessment of the MSC252 *Deepwater Horizon* Oil Spill. NOAA Technical Memorandum NMFS-SEFSC-670. 28pp. doi:10.7289/V5CC0XN0

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Table of Contents

List of Figures

List of Tables

List of Appendices

Introduction

Remote biopsy collection is a sampling technique that was used as part of the Natural Resource Damage Assessment (NRDA) to evaluate impacts from the MSC252 *Deepwater Horizon* (DWH) oil spill on common bottlenose dolphins (*Tursiops truncatus*; hereafter bottlenose dolphins or dolphins) and other marine mammals found in the northern Gulf of Mexico (GMx). Remote biopsy sampling is an effective method to collect skin and blubber samples from free swimming cetaceans for multiple types of analyses (see Noren and Mocklin 2012 for a review): skin for genetic (Sellas *et al*., 2005; Adams and Rosel 2006; Rosel *et al*., 2009; Vollmer 2011), genomic (Mancia et al. 2014) and stable isotope analyses (Herman *et al.*, 2005); blubber to determine contaminant concentrations (Hansen *et al*., 2004; Kucklick *et al*., 2011; Litz *et al*., 2007) and for hormone analysis (Kellar *et al*., 2006); and skin and blubber together to determine CYP1A expression as a biomarker for PAH exposure, as well as for histopathology and immunohistochemistry analyses.

Remote biopsy sampling causes minimal disturbance to the target animal and group. Most studies using remote sampling techniques on small cetaceans have documented that reaction to biopsy sampling is immediate and abbreviated (< 1 min), with no long term behavioral effects observed (Weller *et al*., 1997; Gorgone *et al*., 2008; Kiszka *et al*., 2010; Tezanos-Pinto and Baker 2011). Although remote biopsy collection is a standardized methodology for tissue collection, this sampling technique is not without risk. For example, in the Mediterranean Sea, Bearzi (2000) reported the direct mortality of a short-beaked common dolphin (*Delphinus delphis*) following a remote sampling event.

To assess impacts from the DWH oil spill, remote biopsy sampling of cetaceans was initiated as part of the NRDA in selected bays, sounds, estuaries (BSE), and coastal and offshore waters of the GMx. Offshore waters sampled were within the U.S. Economic Exclusive Zone (EEZ). All samples were collected under Marine Mammal Protection Act Permit numbers 779-1633-02 and 522-1785. Anticipated analyses supported by this sampling method included:

1. Skin for genetic, genomic and stable isotope analyses
2. Blubber for contaminant and hormone analyses
3. Skin and blubber for CYP1A and immunohistological analyses
4. Dorsal fin photographs for individual dolphin identification

Survey Areas

Bay, Sound, Estuarine and Coastal Areas

Initially, five survey areas known to be inhabited by bottlenose dolphins were selected for remote biopsy sampling: Barataria Bay (*e.g.*, Miller 2003) and Chandeleur Sound, LA (*e.g.,* Mullin 1988), Mississippi Sound, MS (*e.g.,* Hubard *et al.* 2004), and St. Joseph Bay, FL (*e.g.,* Balmer *et al.* 2008). These were chosen as areas that could potentially be impacted by the MSC252 DWH oil spill. In summer 2013, dolphins inhabiting the coastal waters of Louisiana, adjacent to Barataria Bay, were also sampled.

Offshore Areas

In summer 2010, a large vessel survey was conducted aboard the NOAA Ship *Gordon Gunter* (*Gunter*). During Leg 1, tracklines were followed in the northwestern GMx between the 200- and 400-m isobaths, within known Bryde's whale (*Balaenoptera edeni*) habitat (Martinez *et al*. 2010). During Leg 2, the focus switched to sperm whale habitat in the north-central GMx along the 1,000-m isobath. Leg 3 of the summer survey was dedicated to sub-surface oil-monitoring, so marine mammal sightings were opportunistic.

In fall of 2010, another large vessel survey was conducted aboard the *Gunter*. Target species for biopsies was primarily Bryde's whales, but other species were sampled opportunistically during the survey including short-finned pilot whales (*Globicephala macrorhyncus*), Atlantic spotted dolphins (*Stenella frontalis*), spinner dolphins (*Stenella longirostris*) and common bottlenose dolphins (Martinez *et al*. 2011). Leg 1 was conducted along the shelf break (200 – 400m depth) in the northwestern GMx. Leg 2 was dedicated to mid-water trawling in the north-central GoM in waters 1,000- to 2,500-m deep.

Mechanics of Biopsy Sampling

For the DWH NRDA, two different devices were used to collect remote biopsy samples from cetaceans: a modified rifle (*e.g.*, John Geiges, South Carolina, USA) (Figure 1) and a crossbow (*e.g.*, Barnett Panzer V, 68kg draw weight, Barnett Outdoors, LLC, Tarpon Springs, FL USA) (Figure 2). Both types of projectors shoot darts fitted with a stainless steel sampling tip (*e.g.*, Ceta-Dart, Copenhagen, Denmark) with an externally beveled sharpened cutting edge and prongs or tabs angled down and inward to hold the sample in place.

During large vessel surveys, biopsy sampling was conducted from either the bow of the ship or from a small boat (7m) deployed from the ship. From the bow of the ship, biopsy samples were collected from small bow-riding cetaceans using rifles and tethered darts. From the small boat both rifles and crossbows were used to deploy untethered darts. Different size sampling tips were used depending on vessel type and target species, including (bore diameter x length in mm): 7x25mm, 7x40mm, 10x25mm and 10x35mm. Typically 7x25mm tips were used from the bow of the ship on smaller cetaceans (*e.g.*, common bottlenose dolphins, Atlantic spotted dolphins). The larger diameter sampling tips (10x25mm) were used when sampling smaller cetaceans from small vessels. The longer length tips were used for larger cetaceans (*e.g.*, Bryde's whales, sperm whales, pilot whales).

Modified rifle

Rifle darts consisted of a graphite or carbon fiber shaft with a sampling tip at one end and a metal tailpiece at the other. The tailpiece slid onto the shaft and was affixed with glue or epoxy. A rubberO-ring [*e.g.,* #7 O-ring (Danco, Irving, TX)] fit into a groove in the metal tailpiece and provided a seal between the dart and the bore. If necessary, another O-ring [*e.g.,* #10 O-ring (Danco, Irving, TX)] was added to the middle of the shaft to allow for stabilization of the dart in the barrel and help the dart maintain a straight trajectory upon firing.

Figure 1. Modified rifle and dart used for remote biopsy sampling during the Natural Resource Damage Assessment of the MSC252 *Deepwater Horizon* Oil Spill.

The sampling tip was threaded onto a metal stopper glued to the other end of the shaft. On the stopper, below the threads, was a flange, 30mm in diameter, which prevented the dart from over-penetrating and aided in the dart rebounding after impact. A closed cell foam mold, for flotation, encased the flange and tapered approximately 10cm down the upper shaft. The sampling tip was designed to collect a small core of tissue that consists of skin and a full-thickness section of blubber (approximately 0.7-0.8g in weight). The dart was discharged from the rifle by means of a blank .22 charge (*e.g.,* Ramset 32CW power level 3 green powder load, ITW Ramset, Glendale Heights, IL). The rifle was equipped with a battery-powered electronic sight to increase sampling accuracy.

Crossbow

Crossbow darts were compatible with the sampling tips used for rifle darts and only differed in design from the rifle darts to accommodate the different mechanics of the two devices. For example, O-rings were not needed for crossbow darts and the metal tailpiece of the rifle dart was replaced with a three feathered fletching and a nock positioned so that one feather of the fletching slid into the groove on the crossbow. A retainer on the crossbow held the dart in place. Also, charges were not needed with a crossbow, instead the string was manually cocked into place until the trigger was pulled to release the string and propel the dart. The crossbow was also equipped with a battery-powered sight to increase sampling accuracy.

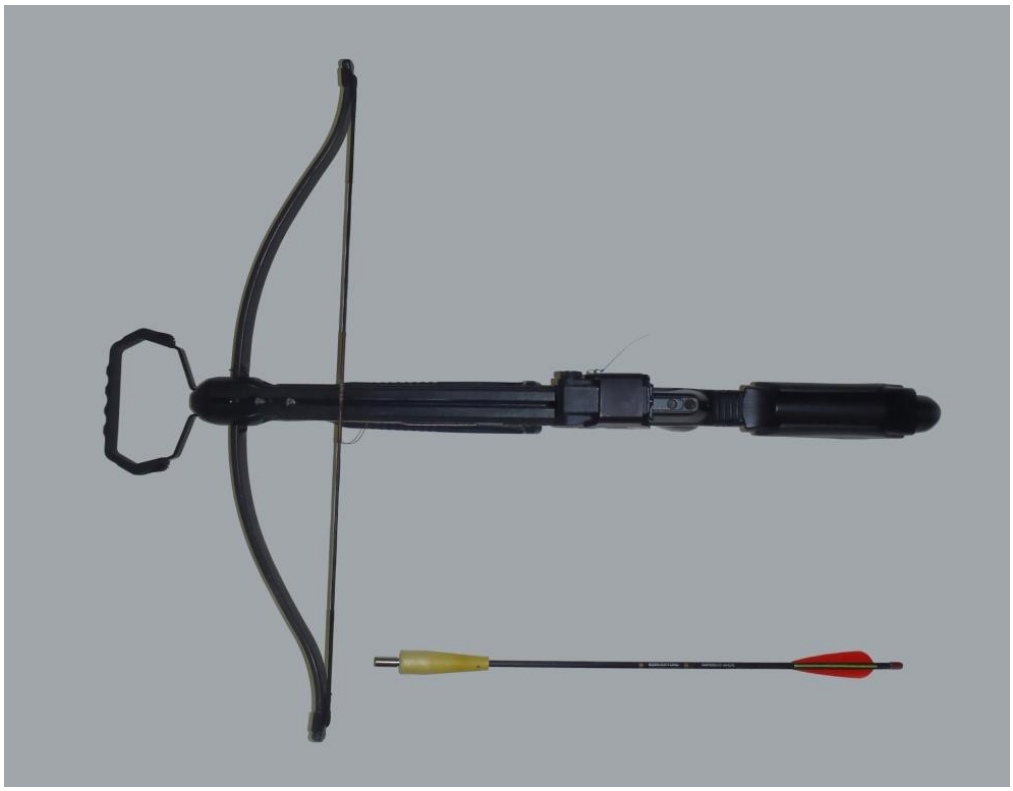

Figure 2. Crossbow and dart used for remote biopsy sampling during the Natural Resource Damage Assessment of the MSC252 *Deepwater Horizon* Oil Spill.

Safety

1. Only personnel with the required training and clearance (*i.e.*, co-investigator on an MMPA permit) were permitted to fire the sampling devices.
2. The sampling devices should have the safety on at all times except when a shot is imminent.
3. The sampling devices should never be pointed in the direction of a human being.
4. A charge should not be placed in the rifle until after the dart has been positioned in the barrel.
5. Unspent charges should never be left inside an unattended rifle.
6. The crossbow should never be "dry-fired" (*e.g.*, if no biopsy shot was taken, the string can be manually un-cocked using a stringer).

Required Survey Equipment

Data Recording

Data recording equipment included a handheld Global Positioning System (GPS) unit and a clipboard containing the following items:

1. Data sheets (printed on waterproof paper):

a. BSE and Coastal Surveys: Survey and Sighting Forms (Melancon *et al.*, 2011), and *Deepwater Horizon* Oil Spill Biopsy Sheets (Biopsy Sheet) (Appendix A)
b. Offshore Surveys: Sighting Form (Appendix B) and Biopsy Sheet (Appendix A)

2. Waterproof pens (2)
3. Spare batteries for the GPS

Sample Processing Kit

Each small boat was equipped with a Sample Processing Kit that usually consisted of a standard-sized cooler (*e.g.,* Igloo Marine 72 quart cooler) for protection from the weather containing the following items (aboard the ship, sample processing occurred in an onboard lab, but the same items were used):

1. Tackle Box:
    a. Sampling implements : stainless steel scalpel handles, forceps and probes
    b. Scalpel blades (No. 10, sterile, stainless steel)
    c. Sample vials (5mL Teflon, 7mL 20% DMSO saturated with sodium chloride (NaCl), 7mL with 3mL of 10% formalin, 5mL RNAlater (after February 2012) and 2mL cryo-vials)
    d. Cryo pens
    e. Sharpies
    f. Small ruler (for measuring sample size)
2. Powder-free Nitrile gloves (multiple sizes)
3. Glass cutting board
4. Heavy duty aluminum foil
5. Small container with lid for holding used implements and sampling tips
6. Plastic sealable bag labeled “waste”
7. Sharps container for scalpel blades
8. Paper towels for drying dart (if necessary)

Vapor Shipper

On the small boat, a small vapor shipper (*e.g.,* MVE Cryoshipper SC 4/2v) was used for storage of cryogenic samples. The vapor shipper was charged with liquid nitrogen at least 24 hours prior to field work, or per the manufacturer’s recommendations. Any excess liquid nitrogen was poured out of the vapor shipper prior to placing samples inside. At the close of daily field operations, samples were transferred from the small vapor shipper to an ultra-low (-80°C) freezer or a large vapor shipper (*e.g.*, MVE Cryoshipper QWIK 10/950). Vapor shippers were re-charged at a liquid nitrogen facility (*e.g.,* AirGas) approximately every 10-14 days during sampling or at the National Institute of Standards and Technology, Charleston, SC between sampling sessions. On the large vessel, cryogenic samples were stored in a -80°C freezer.

Sampling Devices

1. Rifle: The modified rifle was stored inside a waterproof rifle case and was removed from the case only when a biopsy attempt was imminent. Additionally, the rifle required the following items:

a. Charges: Charges for the rifle were kept in an airtight container. Silicone desiccant packets were used in the container to absorb any moisture that could permeate the charges.
b. Rifle cleaning kit: Occasionally the rifle needed to be cleaned while on the small boat. The decision to take the rifle cleaning supplies on the small boat was at the discretion of the sampler.
c. Spare batteries for the electronic sight.

2. Crossbow: The crossbow was secured to the frame of the boat using a bungee cord. The crossbow was strung but not cocked and the safety remained on. Additionally the crossbow required the following items:
    a. Crossbow kit containing spare string and bowstring wax
    b. Spare batteries for the electronic sight

Camera

High resolution, digital SLR cameras were used for photographic data collection. The camera was stored in a waterproof case that contained the following:

1. A 100-400mm or 70-300mm zoom telephoto lens for dorsal fin and/or dart impact photo (Figure 3).
2. A 28-135mm, or similar, macro lens for GPS photos and biopsy sample photos (Figure 4).
3. Multiple 4GB, or larger, CompactFlash or SD memory cards (depending on the camera).
4. At least two spare camera batteries (in addition to the one in the camera).
5. Clean lens cloth(s).

Figure 3. Acceptable dorsal fin images, both right and left sides, and an ideal dart impact image.

Synopsis of Operational Duties

Remote biopsy sampling of BSE and coastal bottlenose dolphins was conducted from small vessels. Sampling of offshore cetacean species was conducted from the bow of the ship or from a small vessel launched and retrieved from the ship. There were four primary roles onboard each small vessel: boat operator, data recorder, photographer, and sampler. A minimum survey crew consisted of at least three scientists, but four crew members were optimal. From the bow of the ship a sampler, data recorder and sample processor were recommended.

Figure 4. Acceptable GPS and biopsy sample photos for the Natural Resource Damage Assessment of the MSC252 *Deepwater Horizon* Oil Spill. Biopsy sample photo illustrates proper set up of sample processing surface in the order of cutting board, ruler (removed after photo), Teflon bag, then sample as described in Appendix C. The seam of the Teflon bag is visible roughly 3cm to the right of the biopsy sample.

Boat Operator

Boat operators were proficient at approaching and safely maneuvering around dolphins to facilitate efficient and effective biopsy sample and photograph collection. In addition, NOAA small boat operators were required to be certified in CPR and First Aid, and also completed the NOAA Small Boat Component Course, as well as a USCG certified Boater's Safety course. Before each daily survey, it was the operator's responsibility to notify the appropriate authorities (*e.g.,* U.S. Coast Guard, state and/or local marine patrols) and fill out a NOAA Small Boat Float Plan. The operator prepared the vessel for each working day. This included ensuring the vessel was fueled and cleaned, and the appropriate boat and safety gear were onboard and in good, workable condition. The operator accounted for each trip in each vessel's log book. The operator was responsible for the safe operation of the vessel and all embarked personnel while underway including ensuring each person aboard their vessel wore an approved personal flotation device (PFD) in accordance with the NOAA Small Boat Standards and Procedures manual. At the end of the day, the operator refueled and cleaned the vessel, and made sure all of the operating and safety gear was cleaned, dried (or drying), repaired (if necessary), and stored in the appropriate area.

Additionally, for small vessels deployed from the ship, the operator was proficient with at-sea deployment and retrieval methods. Besides the normal small boat operator requirements listed in the previous paragraph, boat operators deploying from the ship also completed Fast Boat Rescue Training. Before each deployment, it was the operator's responsibility to work with the ship's command to assess conditions and obtain approval to deploy. While at sea, the ship's engineering department was responsible for refueling the small vessel.

Data Recorder

The data recorders were responsible for accurately, thoroughly and legibly recording data from dolphin sightings and biopsy attempts. At times, the recorder assisted the sampler during sample processing by labeling sample vials with the appropriate sample numbers. At the end of the survey day, recorders were responsible for finalizing data sheets and restocking data sheets and pens for the next survey day. The recorder downloaded track lines and waypoints from the handheld GPS (Appendix D), and ensured that waypoints recorded on sighting sheets were accurate.

Photographer

The photographers' duties included collecting high quality dorsal fin images for photographic identification (photo-ID) purposes, as well as documenting biopsy attempts (Figure 3). They kept track of individuals in the group, and ensured as many high quality images of group members as possible were obtained. They also kept track of individual dolphins that had already been sampled to avoid duplication. After each sampling trip photographers were responsible for downloading images to external hard drives (Appendix E), double-checking (in cooperation with the Data Recorder) that data sheets accurately corresponded to images taken, and cleaning and preparing cameras for use the next working day.

Sampler

The samplers were responsible for obtaining biopsy samples of dolphins while maintaining a safe working environment, which included ensuring safety precautions were adhered to at all times. The sampler was usually responsible for processing the samples (Appendix C), ensuring all sub-samples were labeled and stored appropriately, and coordinating with the data recorder to complete the Biopsy Sheet (Appendix A). Sampler's end-of-the-day responsibilities included proper cleaning and storage of sampling devices, and cleaning (Appendix F) and restocking sample collecting and processing equipment.

Biopsy Survey Methods

During BSE and coastal small boat surveys, biopsy survey methods were similar to photo-ID survey methods described in Melancon *et al.* (2011). However, during these biopsy surveys, tracklines were typically not followed while surveying for groups of dolphins. Melancon *et al.* (2011) also describe completion of the Survey Form, Effort Log, and Sighting Forms used during remote biopsy sampling. For offshore ship surveys, animals were usually approached as part of the species identification and enumeration process. Smaller cetaceans that ride the bow of the ship were sampled from the bow during the normal course of operations. Larger cetaceans that do not bow ride were sampled from a small boat launched from the ship at the discretion of the Chief Scientist and Ship's command. The following methods are more descriptive of methods used during BSE and coastal small boat surveys, but the methods for the offshore surveys are similar.

When a group of dolphins was sighted, it was approached for data collection and to determine suitability for sample collection. Circumstances that would potentially eliminate a group from consideration include: presence of one or more neonates or very young calves (< 1 year old); stress reactions by one or more dolphins; and group primarily composed of previously sampled dolphins. If the group proved suitable, photo-ID operations commenced while the sampler prepared the sampling device, un-wrapped a dart and rinsed it in ethanol. Photo-ID continued while biopsy approaches were underway. However, while complete group coverage is ideal, photo-ID was not the primary objective during remote biopsy sampling.

When the sampler was ready for a sampling attempt and a shot was imminent, the photographer ceased photo-ID sampling of the group and focused on the same dolphin as the sampler. At this time,

documenting the impact of the dart as well as the dorsal fin of the target dolphin was the primary responsibility of the photographer. Shooting may disrupt the groups' behavior and they may become unapproachable, making it difficult or impossible to get a dorsal fin image of the target dolphin post-shot.

To collect a biopsy sample from a cetacean from a small boat, the sampler aimed at the flank of the dolphin below the dorsal fin (Gorgone *et al.* 2008) and above the midline. The target animal should be perpendicular to the sampler when the shot is fired, so the dart impacts the dolphin as flush as possible. From the bow of the ship, the biopsy should be collected from an area near the middle of the animal near the dorsal fin and well behind the blowhole. With the larger 10x25mm sampling tips, oblique dart impacts lowered chances of sample extraction and retention. Sampling distances were typically 3-10m. When a shot was taken, the data recorder recorded a GPS waypoint and completed the Biopsy Sheet (Appendix A). A Biopsy Sheet was required whether or not a sample was collected.

After a shot was taken, the dart was retrieved as efficiently and safely as possible. During small boat surveys, the floating untethered dart was retrieved by hand from the water's surface. From the ship, the dart was tethered to the bow to allow for retrieval. If the shot resulted in a hit, the dart was retired for the day whether or not a sample was collected, to avoid any cross-contamination of samples. If the shot missed, the dart was reused if it only contacted water. The dart was retrieved, the shaft dried, and the sampling tip rinsed with ethanol prior to reloading. If there was any question whether or not the dart struck the target dolphin, it was not reused to avoid possible cross-contamination with a subsequent sample.

Following successful dart retrieval where a sample was present, the sample was processed as quickly and as meticulously as field conditions allowed (Appendix C). During sample processing, the driver and recorder completed the biopsy sheet and kept an eye on the target group to observe post-biopsy behaviors and facilitate possibly collecting additional samples (if applicable). After the sample was processed, sample vials were labeled, recorded and stored, and Biopsy Sheet was completed, another biopsy attempt could be made if the dolphin group was still approachable. No more than two samples were collected and usually, no more than three sampling attempts were made from each group. At the completion of all biopsy attempts from the group, the sighting sheet was finalized and survey effort recommenced.

During BSE and coastal surveys, only 10x25mm tips were used. A full-depth sample yielded six sub-samples. If the sample was not full-depth, the contaminant and hormone sample were combined and placed in a Teflon vial, yielding five samples total. For glancing shots that collected only skin, priority was given to genetic samples, but if enough skin was present, skin for stable isotope analysis was collected as well. Specific procedures for sample processing and storage by tip size are as follows:

During BSE and coastal surveys:

1. 10x25mm

a. $^1/_3$ of total skin, stored in vial with 20% DMSO saturated with NaCl stored at room temperature (genetics)
b. $^1/_6$ of total skin, cryo-vial frozen at -80°C (stable isotopes)
c. $^1/_4$ total skin attached to $^1/_4$ total blubber, cryo-vial stored at -80°C or vial with RNA*later*™ (CYP1A or genomics)
d. $^1/_4$ total skin attached to $^1/_4$ total blubber stored at room temperature in a vial with 10% formalin (immunohistochemistry)
e. $^1/_6$ total blubber, stored in cryo-vial frozen at -80°C (hormones)
f. $^1/_3$ total blubber, stored in Teflon vial frozen at -80°C (contaminants)

During offshore surveys, sample size and number of sub-samples varied depending on sampling tip:

1. 7x25mm
    a. 1st sample from a group
        i. All skin, placed in vial with 20% DMSO saturated with NaCl stored at room temperature (genetics)
        ii. All blubber, stored in a Teflon vial stored at -80°C (contaminants)
    b. 2nd sample from a group
        i. ¼ of all skin, stored in a vial with 20% DMSO saturated with NaCl stored at room temperature (genetics)
        ii. ¼ of all skin attached to ½ of all blubber, stored in at room temperature in a vial with 10% formalin (immunohistochemisty)
        iii. ½ of all skin and ½ of all blubber, cyro-vial stored at -80°C (CYP1A, genomics, or stable isotopes)
2. 10x25-35mm
    a. ½ of all skin, placed in a vial with 20% DMSO saturated with NaCl stored at room temperature (genetics)
    b. ½ of all blubber, Teflon vial and stored at -80°C (contaminants)
    c. ¼ of all skin attached to ¼ all blubber, cryo-vial stored at -80°C (CYP1A, genomics, or stable isotopes)
    d. ¼ of all skin attached to ¼ all blubber, stored in jar with formalin (immunohistochemisty)
3. 7x40mm
    a. ½ of all skin, placed in a vial with 20%DMSO saturated with NaCl stored at room temperature (genetics)
    b. ½ of all blubber, Teflon vial stored at -80°C (contaminants)
    c. ½ of all skin attached to ½ of all blubber, cryo-vial stored at -80°C (CYP1A, genomics, or stable isotopes)

Post Survey Data and Sample Storage Procedures

For estuarine and coastal small boat biopsy effort, all equipment was offloaded from the boat after returning to the dock each day, and each member's post-operational duties commenced. Completed data sheets and mirrored hard drives containing electronic data remained in the possession of the lead sampler until data intake. For both ship and small boat surveys, samples requiring storage at room temperature were transferred to storage boxes and remained in the custody of the lead sampler (or Field Party Chief on the ship) until shipment. Depending on which survey area was sampled, one of two procedures for storage of cryogenic samples was used:

1. Mississippi Sound survey area: The onboard vapor shipper was brought from the dock to the NMFS Pascagoula Laboratory each day samples were collected. The frozen samples were transferred to cardboard freezer boxes and placed in an ultralow (-80°C) freezer which was locked at all times.
2. Barataria Bay, Chandeleur Sound and St. Joseph Bay survey areas: Samples were transferred from the onboard vapor shipper to the medium, "stay-at-home" vapor shipper which was in the possession of the lead sampler. Upon conclusion of field activities, the vapor shipper was transported back to the NMFS Pascagoula Laboratory (Barataria Bay or Chandeleur Sound) or Mote Marine Laboratory (St. Joseph Bay).
3. For large vessel surveys, cryogenic samples were stored in a -80°C freezer on board which was locked at all times. When the ship returned to port, all samples were transported by the lead sampler to the NMFS Miami Laboratory.

At the end of each sampling day, NRDA Sample Collection Form and NRDA Chain of Custody Forms were created for each vessel. These forms were provided by NOAA and located on the *Deepwater Horizon* Information Management Portal (http://www.noaanrda.org/) and instructions for the forms can be found on that website (NOAA/NRDA 2012).

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Appendix A. *Deepwater Horizon* Oil Spill Response Biopsy Sheet

Field descriptions:

Date: YYYY/MM/DD

Assessment stage: Check one

Shot #: Daily consecutive number

Time (shot): Military time to the nearest minute when the shot was fired; associated with WPT

Time (in LN2) Military time to the nearest minute when sub-samples were placed in the vapor shipper

Sighting #: Daily consecutive number; corresponds to Sighting Form

HIT/MISS: Result of SHOT; circle one

Species: Scientific name of species sampled

Platform: initials (≤3 characters), NOAA Ship Gordon Gunter = GU, NOAA Ship Pisces = PC, R/V Relentless III = R3, R/V Typhoon = TYP, R/V Trailing Edge = TE, R/V Relentless II = R2, R/V Top Notch = TN, R/V Boston Whaler = BW

Latitude/Longitude: sample location recorded in decimal degrees (DD.DDDD); associated with WPT

Recorder: Data recorder's initials

Device: Sampling device initials; cross-bow (CB) or dart gun (DG)

Distance at darting: The estimated distance in meters of the target animal from the research vessel

Sampler: Shooter's initials

Sampling Head: Circle one; 1 = Geiges 25mm, 2 = Finn 25mm, 3 = AllTec 30mm

Charge Level: brown (B), green (G), yellow (Y), red (R)

Sample location: Check appropriate location on the left corresponding to area hit on the dolphin (Table 1); mark location hit with an "x" on the drawing to the right

Sample Processing: check each box that applies (Table 2)

Sample #: unique number in vessel, year, month, day, number format; VVYYMMDD-nn; (e.g., GU090630-02; GU = Gordon Gunter, 090630 = 30 June 2009, 02 = sample #2)

Photos (Y/N): circle proper letter corresponding to whether photos were collected during the encounter; space available for up to two cameras

Camera: camera model (e.g., Canon EOS 60D = 60D)

Frame (start): frame from target animal when initial surfacing occurred

Frame (hit): frame capturing dart impact

Frame (end): frame containing sample (after it was removed from the sampling tip) and ruler

Group size: Best estimate of number of adults and calves in the targeted animal's subgroup

Pre-biopsy: What the group was doing when initially sighted, prior to approaching group;

Post-biopsy: What the group is doing after the biopsy attempt,

Target Animal Biopsy Behavior Response(s): Immediate reaction of target animal to biopsy attempt; use Biopsy Behavior Codes on back of sheet, top left (Table 3)

Level of Response: Circle one; Categorizes target animal's strength of reaction to biopsy attempt as None, Low, Moderate, or Strong; Subjective; corresponds to Target Animal Biopsy Behavior Response(s)

Estimated dolphin size: Estimated length of target animal (S, M, L, XL)

Animals Incidentally Harassed: number of animals other than the target animal that reacted to biopsy or biopsy attempt (i.e., if you shoot at 1 animal within a group of 5 animals and all 5 animals react to the biopsy attempt, then 4 animals were incidentally harassed)

Behavior responses: Immediate reaction of animals incidentally harassed to biopsy attempt

Comments: Notes related to biopsy collection or sample processing

Table 1. Description of codes corresponding to location sampled on Target Animal from *Deepwater Horizon* Oil Spill Response Biopsy Sheet.

Code	Abbreviation	Description
1	Head	Head
2	Pec.Fin	Pectoral fin, indicate side in Notes
3	L. Ant. DF	Left side of animal, anterior to dorsal fin
4	R. Ant. DF	Right side of animal, anterior to dorsal fin
5	L. Pst. DF	Left side of animal, posterior to dorsal fin
6	R. Pst. DF	Right side of animal, posterior to dorsal fin
7	Glance L.	Glancing shot on left side of animal
8	Glance R.	Glancing shot on right side of animal
9	Ant. Caud. P.	Dorsal side of animal, anterior to caudal peduncle
10	Caud. P.	Caudal peduncle
11	Flukes	Flukes
12	Belly	Belly
13	Near Blowhole	Near blowhole
14	Below DF L.	Left side of animal, just below dorsal fin
15	Below DF R.	Right side of animal, just below dorsal fin
16	Other	Any other location not described; elaborate in Comments
17	DF	Dorsal Fin
18	Midline Ant. DF	Dorsal side of animal between dorsal fin and blowhole

Table 2. Sub-sample tissue type, intended analysis, and storage method from *Deepwater Horizon* Oil Spill Response Biopsy Sheet.

Tissue type	Intended Analysis	Disposition
Skin	Genetics	Sample vial, 20% DMSO saturated with NaCl, stored at room temperature, in the dark
Blubber	Contaminants	Teflon vial, frozen at -80°C
Blubber	Reproductive and Stress Hormones	Cryo-vial, frozen at -80°C
Skin and blubber	Histopathology and immunohistochemistry	Pre-filled 7mL 3% formalin vial, stored at room temperature
Skin and blubber	CYP1A	Cryo-vial, frozen at -80°C or RNAlater™
Skin (Other)	Stable isotopes	Cryo-vial, frozen at -80°C

Table 3. Biopsy Behavior Codes and descriptions used on *Deepwater Horizon* Oil Spill Response Biopsy Sheet (For each Observed Response, the Level of Response can vary).

Code	Observed Response	Typical Level of Response
00	No response	None
01	Target animal left bow/area	Moderate-Strong
02	Entire group left bow/area	Moderate-Strong
03	Breach and leave bow/area	Moderate-Strong
04	Breach and remain on bow/area	Moderate-Strong
05	Multiple breaches	Strong
06	Quiver	Low
07	Startle reaction – still approachable	Low
08	Startle reaction – unapproachable	Moderate-Strong
09	Change swim direction	Moderate
10	Tail slap	Moderate
11	Deep dive	Low
12	Roll	Low-Moderate
13	Defecate	Low-Moderate
14	Forceful breath	Low-Moderate
15	Reposition on bow	Low
20	Slowly submerge	Low
23	Accelerate quickly	Low-Moderate
24	Arched back	Low-Moderate
31	Tail kick	Low-Moderate
34	Hesitation	Low-Moderate
51	Check six	Low
99	Reaction not observed/noted	NA
30	Fast dive	Moderate
32	Sharking	Moderate
33	Tense	Moderate

Deepwater Horizon Oil Spill Response Biopsy Sheet

DATE _____ (Year Month Day) ☐ pre-impact ☐ during impact ☐ post-impact

SHOT # _____ TIME _____ (shot) _____ (in LN_2) SIGHTING # _____ HIT MISS (circle one)

SPECIES _____ PLATFORM _____

LATITUDE (in decimal degrees) _____ LONGITUDE (in decimal degrees) _____ RECORDER _____

DEVICE _____ EST. DIST. AT DARTING _____m SAMPLER _____

SAMPLING HEAD (circle one) F1 F2 F3 F4 G5 DART GUN CHARGE LEVEL (circle one) B G Y R

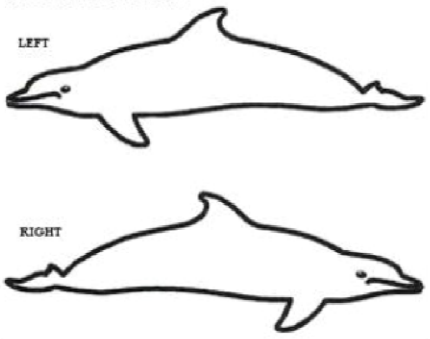
(mark all that apply)

SAMPLE # _____

- ☐ SKIN – GENETICS - DMSO
- ☐ BLUBBER – REPRODUCTIVE HORMONES – FROZEN
- ☐ SKIN & BLUBBER – CYP - FROZEN
- ☐ BLUBBER-CONTAMINANTS-FROZEN
- ☐ SKIN & BLUBBER – CYP - FORMALIN
- ☐ OTHER _____

1 = HEAD	☐	10 = CAUD. P.	☐
2 = PEC. FIN	☐	11 = FLUKES	☐
3 = L. ANT. DF	☐	12 = BELLY	☐
4 = R. ANT. DF	☐	13 = NEAR BLOWHOLE	☐
5 = L. PST. DF	☐	14 = BELOW DF L.	☐
6 = R. PST. DF	☐	15 = BELOW DF R.	☐
7 = GLANCE L.	☐	16 = OTHER	☐
8 = GLANCE R.	☐	17 = DORSAL FIN (DF)	☐
9 = ANT. CAUD. P.	☐	18 = MIDLINE ANT. DF	☐

MARK LOCATION HIT



PHOTOS: YES NO Camera: _____ Frame (start): _____ Frame (hit): _____ Frame (end): _____

Camera: _____ Frame (start): _____ Frame (hit): _____ Frame (end): _____

GROUP SIZE _____ (Adults) _____ (Calves) GROUP BEHAVIOR*: Pre-biopsy _____ Post-biopsy _____

TARGET ANIMAL BIOPSY BEHAVIOR REPONSE* (in chronological order) ☐ ☐ ☐ ☐

LEVEL OF RESPONSE: NONE LOW MODERATE STRONG (circle one) EST. DOLPHIN SIZE _____m

ANIMALS INCIDENTALLY HARASSED _____ BEHAVIOR RESPONSES* (in chronological order) ☐ ☐

COMMENTS: _____

Appendix B. Marine Mammal Sighting Sheet—large vessel

Field descriptions:

Cruise #: unique number in vessel, year, and number format; VVYYnn; (e.g. GU1003; GU = *Gordon Gunter*, 10 = 2010, 03 = third cruise performed by GU in 2010)

Leg #: consecutive number for each cruise; typically 1, 2 or 3

Date: YYY/MM/DD

Sighting #: daily consecutive number

Observer code: unique 2 digit code

Effort: circle one ON/OFF

Biopsy attempt: circle one YES/NO if biopsy attempts were made during the sighting

Observer initials: two or three letter abbreviation

Photos: circle one YES/NO if photographs were taken during the sighting

Biopsy collected: circle one YES/NO if the biopsy attempt(s) was (were) successful

Species 1: Scientific name of species sighted

Species 2: Scientific name of second species sighted in an associated group (if applicable)

Behavior and Reaction to Ship: series of fill in the blank and questions used for permit reporting; use number codes provided and/or circle one appropriate answer; any additional comments can be made in *Comments* field

Were fish associated with the mammal sighting?: circle one and enter species (if applicable)

Were birds associated with the mammal sighting?: circle one and enter species (if applicable)

Comments: notes related to sighting

Multiple Species Sightings: circle corresponding group composition and note any comments on lines provided

Species Description: an observer sketch and verbal description of species sighted; calves and neonates circle one YES/NO/CBD

Additional Comments: notes related to sighting; continued from page 1

MARINE MAMMAL SIGHTING SHEET

Cruise # _____ Leg # _____ Date _____/_____/_____ Sighting # _____
(Year / Month / Day)

Observer Code: _____ Effort: ON OFF Biopsy Attempt: YES NO

Observer Initials: _____ Photos: YES NO Biopsy Collected: YES NO

Species 1_____ Species 2 _____

BEHAVIOR AND REACTION TO SHIP

00 - unknown 02 - resting 04 - complex social 06 - milling 08 - fast travel 10 - other 12 - breaching
01 - slow travel 03 - feeding 05 - bow riding 07 - spy hopping 09 - tail slaps 11 - diving

What was the behavior of the group when initially sighted (list all that apply)? ☐ ☐ ☐

Did the ship approach the group to within 50 yards (for dolphins) or 100 yards (for whales)? YES NO

If yes, did the behavior of the group change when the ship approached to within 50-100 yards? YES NO CBD

If the behavior of the group changed, what was the new behavior (s)? ☐ ☐ ☐

In your opinion, was this group: *attracted to vessel* *fleeing/avoiding vessel* *indifferent* *CBD*

Were fish associated with the mammal sighting? YES NO CBD Species _____

Were birds associated with the mammal sighting? YES NO CBD Species _____

COMMENTS (describe aggregation, movements, and behaviors; please continue on back if more space is needed)

_____

_____

_____

_____

_____

MULTIPLE SPECIES SIGHTINGS

If more than one species of marine mammal was sighted, was the composition of the group:

Mixed *single species subgroups* *both mixed and single species subgroups* *other* *CBD*

Please provide other details that suggested to you the species were associated (e.g., riding the bow together, aggression, one species eating the other, moving in the same direction). If the species were in separate groups/subgroups, please indicate the distance separating them.

_____

_____

_____

Cont. on back

_____

Species Description

List and **sketch** all diagnostic features observed, including estimated body length.

Calves: YES NO CBD Neonates: YES NO CBD

ADDITIONAL COMMENTS - Continued from Page 1

Appendix C. Directions for Processing Samples

1. Begin sample processing using sampling implements cleaned using directions found in Appendix F.
2. Prepared biopsy processing supplies: sampling implements (stainless steel scalpel handle and forceps), scalpel blade (stainless steel, sterile), glass cutting board, small ruler.
3. Prepared sample vials. Number of vials differed depending on vessel and sampling tip, but could include:
    a. 5mL 20% DMSO saturated with NaCLfor genetics
    b. 5mL RNA*later*™ vial for genomics or CYP1A
    c. 5mL Teflon vial for contaminants
    d. 7mL vial with 10% formalin for immunohistochemistry
    e. 2mL cry-vials for hormones, stables isotopes or CYP1A
4. Labeled each sample vial with sample number in vessel, year, month, day, number format; VVYYMMDD-nn (*e.g.*, TE090630-02; TE = R/V *Trailing Edge*, 090630 = 30 June 2009, 02 = sample #2)
    a. In addition to sample number, labeled one cryo-vial with an "H", one with "SI", and one with "CYP" (where applicable)
5. When all processing supplies were ready, donned clean Nitrile gloves
6. Prepared cutting surface. Placed cutting board on a level surface (*e.g.,* on top of Sample Processing Kit) with small ruler on top of cutting board†
    a. Full samples: Opened Teflon bag lengthwise. Tucked ends of the opened bag under the edges of the cutting board, interior-side up (ruler should be between cutting board and exterior of Teflon bag)
    b. Skin-only samples: if sub-sampling was necessary used clean glass cutting board
7. Removed sampling head from dart. Used forceps to remove sample from sampling head and placed on cutting surface
8. Aligned sample with ruler so the Photographer can take a photo of the sample with the ruler to document sample length (mm)
9. Cut sample into sub-samples and placed into appropriate vials
10. Stored various sub-samples
    a. Placed Teflon vial and all cryo-vials in vapor shipper
    b. Replaced DMSO vial and histo vial in Tackle Box

c. Placed RNA*later*™ in cooler with ice

11. Using hemostat or pocket tool (NOT fingers), removed scalpel blade from handle and place into sharps container
12. Placed all used sampling and processing implements (scalpel handle, forceps, sampling tip) in container marked “DIRTY TOOLS”
13. Disposed of used Nitrile gloves, wrappers, and other waste in a re-sealable bag marked “WASTE”

† Beginning in May 2012, samples were processed with the cutting board placed directly on ice to decrease the potential of sample degradation due to high air temperatures

Appendix D. Directions for Downloading Tracklines and Waypoints

1. Attached handheld Garmin GPS to computer via USB cable
2. Turned GPS on
3. Opened MapSource software, located on desktop
4. Selected “Receive from Device” icon, located on toolbar. A window will open asking what data to transfer
5. Checked appropriate boxes for “Tracklines” and “Waypoints” and press “Transfer”
6. Data transferred appeared in the menu box on the left of the screen. Using data sheets, double checked to make sure all data is accounted for
7. Under “File”, selected “Save As”
8. Name file with the following format: YYYY_MMDD_VV (*e.g.,* 2012_0715_TE)
9. Saved the file in Garmin Database format (*.gdb) and GPS Exchange format (*.gpx)

Appendix E. Directions for Downloading Photographic Images

1. Removed compact flash/SD card from camera and insert into appropriate card reader slot
2. Opend the folder containing camera folders on the memory card
3. Copied ALL folders
4. On the NRDA hard drive, created a folder under the current survey (*e.g.*, 2012_05 May MSS Photo ID) in the following format: YYYY_MMDD_Photographer Last_Photographer First_VV. (*e.g.,* 2012_0514_Sinclair_Carrie_TE would be photos from 14 May 2012 taken by Carrie Sinclair on the *Trailing Edge*)
5. Pasted camera photos onto NRDA external hard drive inside the newly created folder – tookseveral minutes
6. Opened the NOAA NRDA Trustees Sampler PhotoLogger Form (PhotoLogger) (NOAA/NRDA 2012)
7. Filled in the fields on the form – note that one PhotoLogger is required for each camera used
8. Saved the PhotoLogger form under the current survey in the following format: YYYY_MMDD_ Photographer Last_Photographer First_VV
9. Copied both the folder containing the photos as well as the completed PhotoLogger form onto the second hard drive (PASC)
10. Removed the compact flash/SD card from the card reader and replace in the camera
11. Formated the compact flash/SD card and manually reset the file numbering on the camera
12. Removde the camera battery and place it on the charger
13. Inserted a fully charged battery into the camera
14. If needed, wiped down camera with an alcohol wipe or soft cloth

Appendix F. Directions for Cleaning Processing Implements and Sampling Equipment

1. Collected necessary cleaning supplies (Table 4)
2. Donned Nitrile gloves
3. Placed all processing implements and sample tips in warm tap water with a small amount of antibacterial soap (*e.g*. Dawn, Aloconox or Chlorhexidine solution)
4. Used toothbrushes to clean processing implements and micro-brushes to clean sampling tips
5. Rinsed well with warm water
6. Used toothbrushes to clean sampling end of dart
7. Placed processing tools and sampling tips in 10% bleach and water solution and let soak (> 10 minutes)
8. Rinsed well with warm water, removing all traces of bleach
9. Rinsed all equipment with fresh water (deionized or reagent grade) followed by 200% ethanol (both in Teflon wash bottles) and allow to thoroughly dry on an aluminum tray.
    a. Covered aluminum tray with a large Kimwipe to prevent dust from settling on processing and sampling implements
    b. In St. Joseph Bay, an autoclave and additional sterilization chemicals (hexane and acetone) were available for cleaning, and all implements after being soaked in ethanol were cleaned using hexane and acetone and then wrapped in foil for high pressure steam saturation
10. When completely dry, wrapped processing implements used for samples containing blubber in ultra-clean Teflon bags (*e.g*. Teflon film bags, KNF Corporation, New York USA).), and replaced in Sample Processing Kit
11. When sample tips were dry, reassemble sampling tip with dart shaft and wrapped entire sampling end in cleaned/sterilized foil (MS/AL, ethanol rinsed; FL, hexane/acetone rinsed), and returned to quiver.
12. Rinsed all cleaning equipment (trays, toothbrushes, etc) and let air dry
13. Disposed of Nitrile gloves and any other waste properly

*Ethanol rinsed, and hexane/acetone rinsed foil was prepared as needed. Heavy duty foil was torn into ~ 5x5” sections. Each piece was cleaned/sterilized and allowed to air dry under a chemical hood (if available).

Table 4. List of supplies needed for biopsy equipment cleaning.

Sampling implements	Cleaning Supplies	Chemicals
Biopsy dart tips	Teflon wash bottles (2)	200% Ethanol
Biopsy dart shafts	Powder-free Nitrile gloves	Bleach
Forceps	Micro brushes	Alconox soap
Scalpel handles	Toothbrushes	Hexane
Probes (optional)	Kimwipes	Acetone
	Heavy-duty aluminum foil	Reagent/deionized water
	Glass jar	
	Aluminum tray	
	Autoclave sterilization pouches	
	Ultra clean Teflon bags	