LCP Dolphin Health Assessment Sampling and Analysis Plan

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1. Introduction

Chronic releases of contaminants from the LCP Chemical Site in Brunswick, Georgia, has continued for over half a century, contaminating the Turtle-Brunswick River Estuary (TBRE), including over 700 acres of salt marsh. Primary contaminants of concern are polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), mercury, and lead. The contamination has impacted benthic biota, as well as birds, fish, shellfish, and mammals, with potentially adverse effects on growth, reproduction, and survival. This Sampling and Analysis Plan (SAP) addresses potential injuries to bottlenose dolphins (*Tursiops truncatus*).

PAHs, mercury, and lead are of concern for bottlenose dolphins, but of particular concern is the PCB contamination from the LCP Site. Because PCBs biomagnify in food webs, apex predators such as bottlenose dolphins can accumulate extremely high PCB concentrations in their tissues. Previous studies documented that PCB concentrations in dolphins from the TBRE are among the highest for marine mammals in U.S. waters (Balmer et al., 2011; Kucklick et al., 2011) and similar to concentrations reported for dolphins in European waters that are associated with long-term population declines not adequately explained by other factors (Jepson et al., 2016).

This SAP includes descriptions of the study objectives, sampling approach, sample collection methods, and documentation procedures. The capture-release health assessment and sampling activities will be conducted under the National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service (NMFS) Permit No. 18786-06 issued to Dr. Teri Rowles and will employ protocols approved by the NOAA/NMFS ad-hoc Institutional Animal Care and Use Committee (IACUC) and reviewed by the Southeast Regional IACUC and NMFS permits office prior to capture fieldwork. In addition to the SAP, a Quality Assurance Project Plan (QAPP) which includes Standard Operating Procedures (SOPs), and a Health and Safety Plan (HSP) have been prepared as standalone documents. The QAPP describes quality assurance procedures such as project organization, sample labelling and data validation. The SOPs describe specific methods, such as laboratory analyses, in detail. The HSP provides guidelines and procedures to protect workers during stated tasks in the SAP.

2. Study Objectives

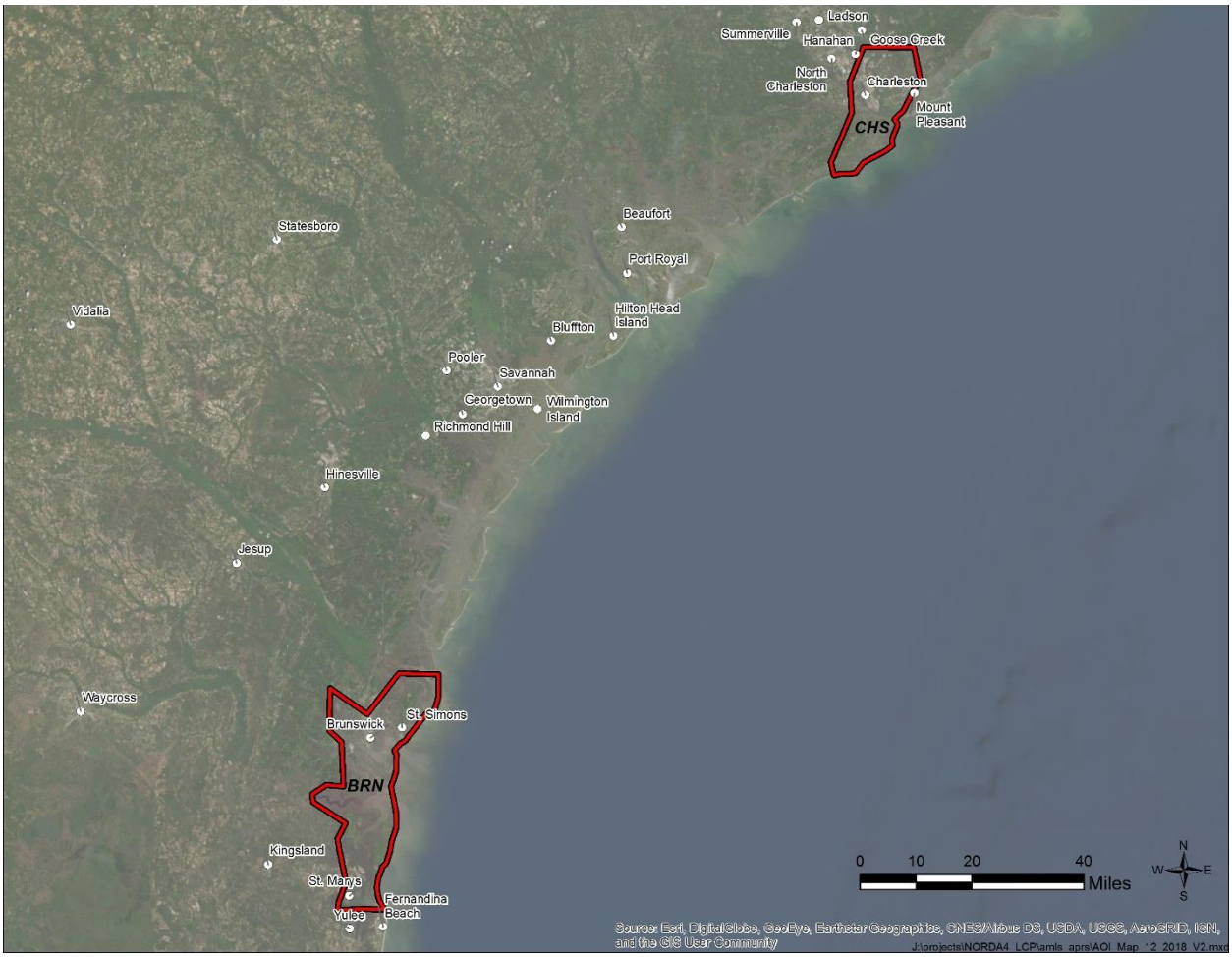
This study will further investigate the potential adverse health conditions associated with chronic PCB exposure for dolphins inhabiting waters near the LCP Chemical Site. The study will conduct clinical health evaluations of bottlenose dolphins near Brunswick, Georgia, and in a comparison site (Charleston, South Carolina) to examine sublethal health impacts from the chronic PCB exposure. The study will involve a team of veterinarians, biologists, and wildlife epidemiologists working together to temporarily capture free-swimming bottlenose dolphins, conduct comprehensive health evaluations, and then release the dolphins onsite. The health assessments will enable researchers to directly assess potential injury endpoints (e.g., anemia, organ damage, immune suppression, endocrine disruption). Additionally, the capture-release operation will allow for attachment of satellite-telemetry tags to dolphins inhabiting the area near the LCP Chemical Site to better understand their small-scale movements, ranges, and preferred habitats, which may assist in both injury quantification and restoration planning. Tags may be deployed on pregnant female dolphins from either the Brunswick or Charleston sites to support future follow-up work to assess reproductive success.

3. Study Design

3.1 Study Area

We have identified two areas (study areas) where health assessments will be carried out for this study (Figure 1). The study areas include the TBRE estuary and surrounding areas (BRN), and the comparison estuarine site around Charleston (CHS).

Figure 1. General field sampling areas for this study and previous studies. The Health Assessment will occur at the BRN and CHS sampling areas.



3.2 Sampling Approach

We plan to conduct health assessments that will entail temporarily capturing, examining, and releasing dolphins from each site during two 2-week periods in June 2022 (CHS) and July 2022 (BRN). Due to their size (400–500 + lbs.), and their protected status under the Marine Mammal Protection Act (MMPA), capturing and restraining dolphins while minimizing risk to the animals and personnel requires specific skills and expertise. NMMF brings extensive experience with capture-release health assessment methods and have led and been involved in many such studies across the southeastern United States (Schwacke et al., 2010, 2014,Hansen et al., 2004; Schwacke et al., 2012; Balmer et al., 2018).

The health assessment will include a complete physical examination including, but not limited to an ultrasound for determination of pregnancy, a diagnostic ultrasound, an examination of eyes, oral cavity, and skin condition; as well as the sample collection and analyses described in Tables 1 and 2. Additional samples may be collected for archiving.

Table 1. Health parameters expected to be measured from dolphin health assessment samples

Blood	
Hematology	Hematocrit, packed cell volume, hemoglobin, red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width, reticulocyte count, white blood cell (WBC) count and differential (segmented neutrophils, lymphocytes, monocytes, and eosinophils), platelet count, plasma appearance, RBC morphology, WBC exam, parasites, and fibrinogen
Electrophoresis	Total protein, albumin, alpha 1 globulin, alpha 2 globulin, total alpha globulin, beta 1 globulin, beta 2 globulin, total beta globulin, and gamma globulin
Serum Chemistry	Electrolytes (sodium, potassium, and chloride), bicarbonate, anion gap, urea nitrogen, creatinine, uric acid, calcium, phosphate, magnesium, total protein, albumin, globulin, glucose, enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST], succinate dehydrogenase [SDH], lactate dehydrogenase [LDH], alkaline phosphatase [ALP], and gamma glutamyl transferase [GGT]), total bilirubin, direct bilirubin, indirect bilirubin, amylase, cholesterol, triglycerides, creatinine kinase, iron, total iron binding capacity, percent iron binding saturation, lipemia, hemolysis, and icterus
Endocrinology	Total thyroxine, free thyroxine, total triiodothyronine, progesterone, cortisol, aldosterone, testosterone, and reverse triiodothyronine
Functional Immunology	T-lymphocyte proliferation, B-lymphocyte proliferation, neutrophil phagocytosis, monocyte phagocytosis, cytokine panel, and regulatory T-cell count
Blubber/Skin	
Chemical analysis	See Table 2
Genetics	Epigenetic age estimation
Aspirate	
Cytology	WBC count and differential, cell morphology, and parasites
Radiography	
Pectoral Fin	Age estimation

Table 2. Chemical contaminants expected to be measured in dolphin blubber samples

∑PCBs	∑DDTs	∑Chlordanes	∑HCHs	∑PBDEs
Congeners: 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 199, 205, 206, 208, 209	o,p'-DDD p,p'-DDD o,p'-DDE p,p'-DDE o,p'-DDT p,p'-DDT	Oxychlordane Gamma-chlordane Nona-III-chlordane Alpha-chlordane Trans-nonachlor Cis-nonachlor	Alpha-, beta-, and gamma-HCH Isomers	Congeners: 28, 47, 49, 66, 85, 99, 100, 153, 154, 183

3.2.1 Procedures for Capture

Dolphins will be captured using long-established methods (Schwacke et al., 2014; Balmer et al., 2018; Barratclough et al., 2019). The capture fleet will consist of six or more boats, including a net boat, three chase boats, a veterinary processing boat, and an auxiliary supply boat. The crew will include a Medical Safety Officer to oversee safety of the operations and to provide emergency medical assistance if necessary. Capture operations will adhere to safe boating regulations and all staff will be briefed on safety protocols prior to going out on the water.

A dolphin group will be followed until it reaches a location where the net (a 365-m long, 6.5-m deep, 22-cm stretch mesh seine net with a float line and a lead line with an internal lead) can be safely deployed, and personnel will be able to safely handle captured dolphins. Our aim will be to capture small groups of dolphins (< 5) in shallow (< 1.5 m) water, over solid to semi-solid substrate, in protected areas with as little current as possible. Alternatively, in deeper (> 2 m) water, one or two dolphins can be captured using a modified technique that does not rely on putting handlers into the water around the net.

In the capture process, the net boat will encircle the targeted dolphins while deploying the capture net astern. The chase boats will be distributed around the compass, maintaining clearance so as not to impede the net boat. Once the compass is closed, chase boat crews will either deploy around the outside of the net (in shallow water) or will remain on their boats (in deep water). If one or more dolphins becomes entangled in the net relatively quickly, handlers can respond to and support these animals. If the encircled dolphins do not become entangled, the net boat and/or dolphin handlers can reduce the size of the compass to force dolphins to entangle themselves. Entangled dolphins will be restrained and supported by handlers, disentangled, and then removed from the net.

Captured dolphins will then be processed, in the water and/or onboard the processing vessel. Procedures are organized to minimize the amount of time an animal spends out of the water. Standardized data collection protocols, established over the course of previous dolphin health assessments, will be used (Wells et al., 2004; Schwacke et al., 2014; Balmer et al., 2018). Each dolphin will be released once it is processed unless there is a reason to release animals together (e.g., a socially dependent mother/calf pair). In such a circumstance, the animals will be processed and held in the water until a dual release is possible. Dolphins that do not adapt to capture and/or sampling may be released early, before the sampling is complete, at the discretion of the veterinarians.

3.2.2 Procedures for Health Evaluation

Blood will be drawn from fluke vessels while the dolphin is held in the water. Mature females (> 220 cm) will then be examined via ultrasound to diagnose their reproductive state. Pregnant animals will be given an abbreviated exam. For early-term pregnancies, a more complete exam may be given at the discretion of the lead veterinarian. Non-pregnant females and males may be placed in a sling, brought aboard the processing vessel, weighed, and either returned to the water or kept onboard for further evaluation. Dolphins kept on the processing vessel will rest on a foam mat and be supported and kept wet by handlers. The complete examination will include sampling of blubber for contaminant analysis. Each dolphin that has not been previously branded or that has been previously branded but the brand has faded will be branded with a three-digit numeric or alphanumeric code on either side of its dorsal fin. These ID numbers provide a long-term

identifier and aid in recognition during monitoring or mark-recapture surveys (Wells, 2009), or in the case of subsequent stranding.

3.2.3 Procedures for Telemetry

Prior to release, some dolphins may receive a single-pin satellite-telemetry tag, which would be attached along the lower third of the trailing edge of the dorsal fin. Specifications and attachment/programming protocols for satellite transmitters have been outlined in multiple previous studies (Balmer et al., 2014, 2018; Wells et al., 2017). Tags will be coated with an anti-fouling material to reduce biogrowth and affixed in such a manner as to minimize drag and irritation on the animal while maximizing the duration of tag attachment. Tags have a projected battery life of 280 days. To increase battery life and provide high-quality location data, tags will be programmed to specifically target transmission windows with optimal satellite coverage.

3.2.4 Procedures for Sample Analysis

Wherever possible, analytical and diagnostic laboratories have been selected based on their records of high quality, as well as their ability to maintain consistency with prior health assessments and allow for comparison with existing reference intervals (e.g., Schwacke et al., 2009). Samples for hematology, clinical chemistry, and serum electrophoresis and endocrinology will be sent to the Animal Health Diagnostic Laboratory (AHDL) at Cornell University for analysis. Functional immunology will be conducted by the laboratory of Dr. Sylvain De Guise (University of Connecticut). Dr. De Guise's laboratory provides expertise in immunotoxicology and extensive experience in the conduct and interpretation of functional immune assays for cetaceans (DeGuise et al., 1995, 1996, 2006; Levin et al., 2004, 2007; Mori et al., 2008; Schwacke et al., 2010).

Analysis of blubber/skin for chemical contaminants and genetic relatedness will be conducted by the NOAA/NMFS Northwest Fisheries Science Center (NWFSC) (e.g., Schwacke et al., 2014) and Southeast Fisheries Science Center (SEFSC) (e.g., Vollmer et al., 2021), respectively. Measurements of the thyroid hormone reverse triiodothyronine will be conducted by Dr. Dorian Houser of the National Marine Mammal Foundation with Sonoma State University (Houser 2021a and b), and evaluation of morbillivirus infections will be assessed by the University of Georgia Athens Veterinary Diagnostic Laboratory (Saliki et al., 2002; Balmer et al., 2018). Ages based on epigenetics and pectoral radiography will be estimated by Dr. Ashley Barratclough of the National Marine Mammal Foundation (Barratclough et al. 2019b and 2021).

4. Documentation and Data Management

4.1 Documentation

Chain-of-custody procedures will be observed for all NRDA samples. All samples will be transferred with appropriate chain-of-custody forms. Survey and sighting data will be recorded using the same forms used during photo-identification surveys (see LCP Dolphin Photographic Survey Sampling and Analysis Plan, 2021). The GPS tracks, scanned data forms, and photos will be loaded to hard drives that are secured and following the trip, they will be loaded to a secure data management system.

For the health evaluations, a separate data packet will be used for each dolphin. Entries will be made with a ball point pen or permanent marker (e.g. Sharpie), and corrections will be made

with a single line through the error with the corrector's initials.

Documentation and sample labelling guidelines are described in detail in the standalone QAPP. Samples, documentation, electronic data collection devices, and other materials will be kept in the possession of the field sampling team at all times in the field. After each day of field sampling, field recorders will review the electronic materials, sample collections, chain of custody forms, and datasheets and make any necessary corrections.

Any deviations from this field sampling plan will be recorded in detail and the rationale for stated deviations will be provided in a project-specific daily log. The field team manager will be responsible for this documentation.

4.2 Data management

A scanned copy of the survey and health evaluation datasheets will be uploaded to the Natural Resource Damage Assessment (NRDA) LCP File Collections in DIVER each evening if possible. The GPS tracklines and photographs, along with scanned survey datasheets (if unable on a given day to be uploaded to DIVER) will be uploaded and kept on two external hard drives (one serving as a duplicate of the other) for archival purposes under chain of custody to transfer to DIVER at the end of the survey. GPS tracklines, photographs, and all scanned survey datasheets will then be uploaded to the NRDA DIVER portal at the end of the survey using one of the archival external hard drives. A third external hard drive containing the same information as on the archival hard drives will be maintained by the NOAA LCP NRDA team for analytical use. All survey datasheets forms will be entered into an electronic database (FinBase) that is maintained by the NOAA LCP NRDA team. This database will undergo quality control (QC) checks to ensure that photograph and waypoint GPS data match the database materials, and that all sighting data were entered accurately.

Data from the health assessment data packets will be entered into electronic spreadsheets and undergo QC checks to ensure that all data were entered correctly. Once laboratory reports are received, these data will also be entered into spreadsheets and undergo QC checks.

Once the QC is complete, the Finbase database and associated files, and the health data spreadsheets will be uploaded to LCP File Collections in DIVER for long-term storage. If any corrections to the sample spreadsheets are required, they will be noted in the database and any previous files will be flagged with the corrections.

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