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Protocols for
Conducting Dolphin Capture-Release Health Assessment Studies

Bottlenose Dolphin Health and Risk Assessment Project

NOAA Technical Memorandum NOS NCCOS 49

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Protocols for Conducting Dolphin Capture-Release Health Assessment Studies

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Introduction

Marine mammals, such as dolphins, can serve as key indicator species in coastal areas by reflecting the effects of natural and anthropogenic stressors. As such they are often considered sentinels of environmental and ecosystem health (Bossart 2006; Wells *et al.* 2004; Fair and Becker 2000). The bottlenose dolphin is an apex predator and a key component of many estuarine environments in the southeastern United States (Woodward-Clyde Consultants 1994; SCDNR 2005). Health assessments of dolphins are especially critical in areas where populations are depleted, show signs of epidemic disease and/or high mortality and/or where habitat is being altered or impacted by human activities.

Recent assessments of environmental conditions in the Indian River Lagoon, Florida (IRL) and the estuarine waters surrounding Charleston, South Carolina (CHS) highlight the need for studies of the health of local bottlenose dolphins. While the condition of southeastern estuaries was rated as fair in the National Coastal Condition Report (U.S. EPA 2001), it was noted that the IRL was characterized by poorer than expected benthic communities, significant sediment toxicity and increased nutrient concentrations. Similarly, portions of the CHS estuary have sediment concentrations of aliphatic aromatic hydrocarbons, select inorganic metals, and some persistent pesticides far in excess of reported bioeffect levels (Hyland *et al.* 1998). Long-term trends in water quality monitoring and recent scientific research suggest that waste load assimilation, non-point source runoff impacts, contaminated sediments, and toxic pollutants are key issues in the CHS estuary system. Several 'hot spots' with high levels of heavy metals and organic compounds have been identified (Van Dolah *et al.* 2004). High concentrations of anthropogenic trace metals, polychlorinated biphenyls (PCB's) and pesticides have been found in the sediments of Charleston Harbor, as well as the Ashley and Cooper Rivers (Long *et al.* 1998). Two superfund sites are located within the CHS estuary and the key contaminants of concern associated with these sites are: polycyclic aromatic hydrocarbons (PAH), lead, chromium, copper, arsenic, zinc and dioxin.

Concerns related to the overall health of IRL dolphins and dermatologic disease observed in many dolphins in the area (Bossart *et al.* 2003) initiated an investigation of potential factors which may have impacted dolphin health. From May-August 2001, 35 bottlenose dolphins died in the IRL during an unusual mortality event (MMC 2003). Many of these dolphins were diagnosed with a variety of skin lesions including proliferative ulcerative dermatitis due to protozoa and fungi, dolphin pox and a vesicular dermatopathy of unknown etiology (Bossart *et al.* 2003). Multiple species from fish to dolphins in the IRL system have exhibited skin lesions of various known and unknown etiologies (Kane *et al.* 2000; Bossart *et al.* 2003; Reif *et al.* 2006). On-going photo-identification (photo-ID) studies have documented skin diseases in IRL dolphins (Mazzoil *et al.* 2005). In addition, up to 70% of green sea turtles in the IRL exhibit fibropapillomas, with the highest rates of occurrence being seen in turtles from the southern IRL (Hirama 2001).

The Bottlenose Dolphin Health and Risk Assessment (HERA) Project, initiated in 2003, is a collaborative effort between the National Ocean Service's Center for Coastal

Environmental Health and Biomolecular Research and Harbor Branch Oceanographic Institution. This comprehensive, integrated, multi-disciplinary research project is designed to assess the health of Atlantic bottlenose dolphins (*Tursiops truncatus*) in CHS and the IRL. Standardized screening-level assessments are performed to meet project objectives, which include the development of tools and techniques to better assess the health of dolphins, identification of threats to dolphin health and investigation of links to possible environmental stressors. Assessments involve the capture, sampling and release of dolphins. Physical examinations are performed and a suite of non-lethal morphologic and clinicopathologic parameters are measured to develop indices of dolphin health. Dolphin health is being evaluated on individual, population and comparative perspectives at the two distinct geographic locations. To facilitate comparisons and minimize seasonal variability between sites, sampling is conducted during periods of similar water temperatures. In addition to developing links to possible environmental stressors, research goals also include developing a better understanding of the cumulative effects of multiple stressors on dolphin health. Research associated with the Dolphin HERA Project will provide information critical to the preparation of effective management plans.

Standardized surveillance/biomonitoring assessments are critical components of programs designed to detect health status and disease processes. In order to assess health trends it is important to use standardized methodology. To enhance the validity of comparisons between the IRL and CHS populations, all methods are standardized using Standard Operating Protocols (SOPs) with Quality Assurance/Quality Control measures. Protocols initially established in 2002 have been modified in subsequent years to incorporate additional evaluations and tests that provide more sensitive methods for measuring health.

Safety protocols were developed for capture-release (C-R) operations at both sites (Appendix A). A review by Norman *et al.* (2004) provides an excellent framework for health concerns to both animals and humans related to C-R studies of odontocetes. The safety protocols developed for the Dolphin HERA Project include guidelines for both human and dolphin safety and provision of on-site medical personnel and safety equipment. Safety measures utilized in the Dolphin HERA Project are continuously reviewed and revised to maximize safety during C-R field operations. Specific items reviewed include: methods to improve net deployment, safe vessel operation, personal safety and a description of emergency support personnel. The current Dolphin HERA Project Safety SOP has been adopted by other groups conducting dolphin C-R studies, such as the Sarasota Dolphin Research Program and the NOAA Fisheries Marine Mammal Health and Stranding Response Program (Schwacke *et al.* 2001).

Methods and procedures being used for the Dolphin HERA Project expand upon previous health assessment studies (Hansen and Wells 1996). Detailed health assessment protocols and forms (Appendix G) were developed to standardize data and sample collection during C-R operations and subsequent sample analysis. Individual dolphin health is evaluated using a suite of tests and measurements including physical examination, hematology, serum chemistry, diagnostic ultrasound, cytological analysis, microbiological, urinalysis, as well as biomarker and contaminant analysis. This report provides a compilation of the Dolphin HERA Project sampling protocols and forms used during C-R studies. The

protocols developed for the Dolphin HERA Project will facilitate a greater comparison of data collected between dolphin projects.

Efficient sampling and field operations are expedited through advance preparation of dolphin sample kits, containing all necessary supplies for sample collection and processing. A boat-borne field laboratory is outfitted to perform sample processing at the time of collection, including blood centrifugation and serum and plasma separation, as well as specific assays which require immediate testing such as erythrocyte sedimentation rate, I-STAT blood analysis, urinalysis, gastric pH and cytologic specimen preparation. An array of temperature storage units (*i.e.*, room temperature, cold, dry ice, liquid nitrogen vapor shipper) are utilized to provide optimum temperature for all samples collected. Specialized mailers and protocols for overnight delivery of samples are essential to expedite and optimize the receipt of high-quality samples for research analyses. The success of the capture and sampling activities depend upon many factors. Establishing a team with a broad range of capabilities and expertise and a well-organized logistical infrastructure and support are important components for conducting effective and safe C-R studies.

Study Sites

Both the CHS and IRL sites have long-term and on-going dolphin photo-ID studies (CHS since 1994, IRL since 1996) and evidence for long-term site fidelity (Zolman 2002; Mazzoil *et al.* 2005; Speakman *et al.* 2006). Photo-ID data can provide information on dolphin residence and distribution patterns which, in turn, can enable a comparison of dolphin health with potential environmental exposures. Sighting data on the distribution, movements and associations of individually identifiable dolphins can assist in the interpretation of data on health, disease and contaminant body burdens.

<u>*Charleston, SC (CHS)*</u> – The study site is located in estuarine waters near Charleston, SC (32º46'51"N, 79º55'33"W), situated in the central region of the state's coastal zone. The site includes the Charleston Harbor, as well as portions of the main channels and creeks of the Ashley, Cooper, and Wando Rivers, and the Stono River Estuary (Fig. 1). The Charleston Harbor-Cooper River coastal plain measures 66 km^2 with an additional 105 km^2 of intertidal wetlands (S.C. Sea Grant Consortium 1992).

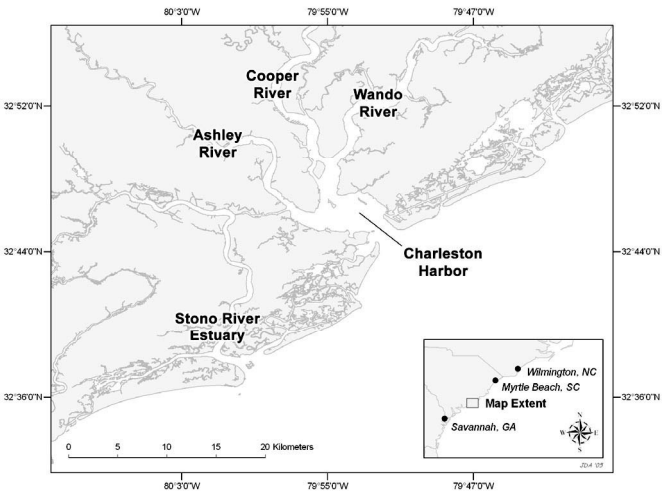

Figure 1. Charleston, SC study site

The Charleston Harbor estuary is the third largest estuarine drainage area in South

Carolina. It is surrounded by urban and residential development and is the fifth largest U.S. port and the second largest container port on the Atlantic seaboard (Tiner 1997). From 1970-1990 the human population grew by 66% in the Charleston tri-county area, which currently totals 500,000 people. It is the fastest growing metropolitan area in South Carolina and an additional 600,000 people are projected by 2015 (U.S. Census Bureau 2000).

The estuary has a soft mud bottom, an average depth of 12 m at low tide and is subject to semidiurnal tides with a mean range of 1.6 m near the ocean (S.C. Sea Grant Consortium 1992). In addition to Charleston Harbor, C-R activities were also carried out in the Stono River Estuary, approximately 20 km southwest of Charleston Harbor. This area is a well-mixed, C-type salt marsh estuary, with little freshwater inflow (Day *et al.* 1989), extensive mud banks and marsh vegetation, and is primarily impacted by residential development.

Indian River Lagoon, FL (IRL) – In the IRL, C-R activities were conducted in two geographic areas (Fig. 2). The northern capture sites included Mosquito Lagoon, and portions of the Indian and Banana Rivers north of latitude 28º15'0"N. The southern capture sites included the St. Lucie Inlet, the north and south forks of the St. Lucie River and the Indian River south of latitude 27º25'0"N.

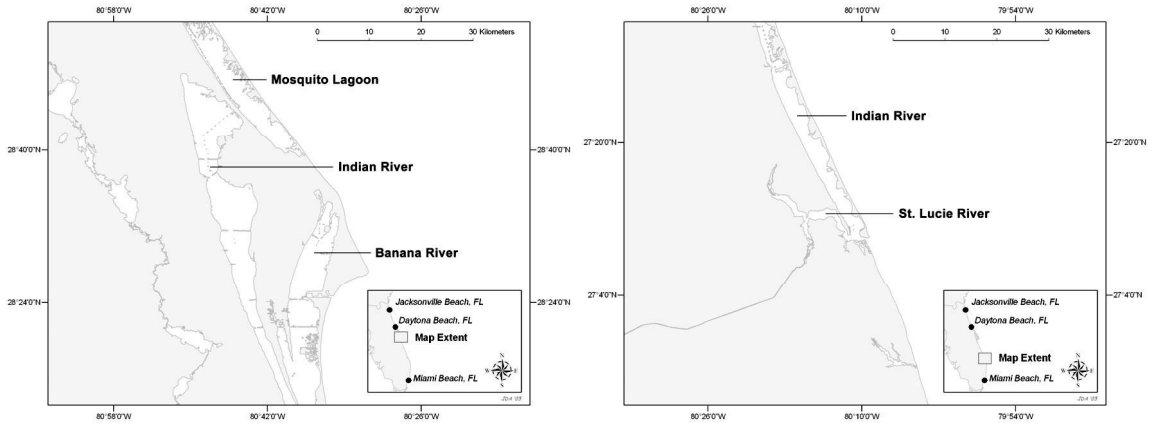

Figure 2. Northern (left) and southern (right) Indian River Lagoon, FL study sites

The IRL is an aggregate of three estuarine water bodies that comprise 40% of Florida's east coast and represents the most biodiverse coastal estuary in North America (Gilmore 1985; IRLNEP 1996). This unique shallow water ecosystem includes the Indian River, Banana River and Mosquito Lagoon and extends 250 km from Ponce De Leon Inlet in the north to Jupiter Inlet in the southern region of the lagoon. The importance of the IRL was emphasized in 1990, when it was named an Estuary of National Significance (IRLNEP 1996). The IRL is an extensive ecosystem spanning two biogeographic zones characterized by temperate marshes and tropical mangrove vegetation. It is comprised of wide shallow lagoons and narrow tidal creeks. The average depth in the IRL is 1 m, although navigation channels are maintained at a depth of 3-4 m. The IRL is connected to the Atlantic Ocean by five small and widely spaced inlets and one lock. Because of the

restricted connections with the ocean, tidal ranges in the lagoon are small, averaging less than 0.3-0.6 m.

Ecosystem concerns specific to the IRL include: degradation of sea grass habitat, alteration of water flow and declining water quality. Dense human development and population growth along the eastern coast of Florida and intense agricultural activities have resulted in increased freshwater inputs and altered water quality (*e.g.*, chemical contamination, high nutrient input, decreased salinity) (IRLNEP 1996). In a lagoon naturally characterized by low water turnover due to the limited number and small size of inlets connecting to the open ocean (Sigua *et al.* 2000), additional impacts may have serious implications for indigenous biota. The lagoon is particularly vulnerable to the influx of pollutants with long residence times because of its limited flushing capacity.

The major cause of water quality decline in the IRL is fresh water runoff and storm water discharge, resulting in altered salinity, decreased water clarity and the introduction of nutrients and pollutants (Scott *et al.* 2002). All of Florida's sugarcane, approximately 38% of the citrus and 42% of the vegetable crops are grown in regions that drain into the IRL (Miles and Pfeuffer 1997). Furthermore, human population growth adjacent to the IRL increased 124% from 1970 to 1990 and is projected to reach 1.1M by 2010 (IRLNEP 1996).

Dolphin HERA Project Summary

In 2003, 2004 and 2005, 194 bottlenose dolphins were captured, examined, sampled, marked and safely released. Annually, C-R field activities were conducted from 7 to 10 days at each site during the summer. Current studies have documented orogenital papillomas in free-ranging bottlenose dolphins from both the IRL and CHS sites, establishing the first reported cases in Atlantic coastal dolphins (Bossart *et al.* 2006). While no lingual or genital papillomas were found in dolphins during the first year of health assessments, an increase in the number of cases occurred over the three year study period (IRL: 0%-2003, 10%-2004 and 47%-2005; CHS: 0%-2003, 8%-2004 and 20%-2005). Preliminary evidence suggests that these tumors may be infectious, most likely via an orogenital transmission route (Bossart *et al.* 2006). Thus, sexual and parturition-associated transmission may be important components in disease pathogenesis. Additionally, the presence of space-occupying genital lesions has potential detrimental implications for successful breeding. Lobomycosis, a chronic mycotic skin disease, was detected in 30% (9 out of 30) of the dolphins captured in the southern IRL but not in any of the 45 dolphins captured in the northern IRL or 71 dolphins captured near CHS during the first two years of health assessments (Reif *et al.* 2006).

The close proximity of large industrial facilities and higher (relative to the IRL) concentrations of various organic contaminants in sediments warrant concern about the health of CHS dolphins. Analysis of blubber samples from CHS dolphins found high levels of legacy contaminants such as PCB and DDE (Hansen *et al.* 2004). Current findings from the Dolphin HERA Project confirm the high concentrations of legacy contaminants, but more importantly, reveal extremely high levels of emergent chemicals of concern. For example, CHS dolphins had higher blood levels of perfluorinated

compounds than any dolphin population surveyed to date (Houde *et al.* 2005). Compared to IRL dolphins, CHS dolphins had significantly higher blubber levels of polybrominated diphenyl ethers representing some of the highest measured in marine mammals (Fair *et al.* in press). In addition to potential impacts from anthropogenic contaminants, other threats to CHS dolphins include fishery interactions. Although the cause of mortality for the majority of bottlenose dolphins stranded in South Carolina are unknown, during 1997-2003 evidence of human interaction was found to account for 25% of dolphin strandings (McFee *et al.* 2007).

Capture-Release Standard Operating Protocols

Dolphin C-R field operations are logistically complex, require 7 or more small boats, a large amount of specialized equipment and a team of 30-60 individuals possessing a broad range of capabilities. Ideally, half of the team should have prior C-R experience. The overall goal of C-R field operations is to capture (Fig. 3), conduct physical examinations, collect samples, mark and release selected dolphins as safely and quickly as possible with a minimum of stress to personnel and to the dolphins.

Figure 3. Capture personnel observing handlers supporting an entangled dolphin

Logistics

Notification – The following agencies are notified in-advance of the commencement of C-R activities associated with the Dolphin HERA Project:

- National Marine Fisheries Service-Southeast Regional Office
- National Marine Fisheries Service Law Enforcement
- United States Coast Guard
- Local law enforcement agencies (state, county, city, etc.)
- Any additional agencies with jurisdiction over, or an official presence in, areas where research activities are planned

Notification should include the following information: a brief description of the research, dates, times and area of planned operations, descriptions of each of the boats involved (including hull ID numbers) and contact information (including the Permit Holder, Principal Investigator, Capture Manager (CM) and Regional Capture Advisor (RCA).

Boats – A minimum of 7 boats, with the capacity to transport all personnel and necessary sampling equipment, are required (Table 1). Fig. 4 provides examples of boats used during the Dolphin HERA Project. Rigid hull inflatable boats make effective Chase

Table 1. Vessels used to conduct HERA C-R operations with suggested number of each needed, crew size, primary role(s) of the vessel and necessary vessel features/capabilities

Vessel	Number	Crew*	Function	Features/Capabilities
Catch Boat	1	3-5	• Transport net • Set/maneuver net	• Shallow draft • Rapid acceleration • Deploy net safely and effectively
Dolphin Processing	1	3-6	• Process dolphin	• Shallow draft • Open working area • Stable at anchor • Davit • Low freeboard • Sun protection (for dolphin)
Sample Processing	1	12+	• Process samples • Transport gear/staff	• Shallow draft • Open deck space • Large storage capacity • Stable at anchor • Sun protection (for staff and samples)
Chase Boat	2+	6-8	• Carry/deploy handlers • Locate dolphins	• Shallow draft • Low freeboard • Maneuverability • Fast
Photo-ID	1	3	• Locate dolphins • Photo-Identification	• Stable • Low engine noise • Good sightlines
Safety	1	3-5	• Transport safety personnel • Emergency Response	• Shallow draft • Storage capacity • Open deck space • Fast

**crew number includes captain and mate*

Boats, because their inflatable sponsons are less rigid than an aluminum or fiberglass hull and provide some degree of cushion to a dolphin brought alongside. Each boat captain should designate an alternate boat operator, capable of all carrying out all necessary boat operations (driving, communicating on the radio, anchoring, etc.) in the captain's absence. Boats should be equipped with the following:

- USCG required safety equipment:
  - * Personal Flotation Devices (PFD) - enough for each member of boat crew
  - * Throwable float
  - * 1-2 Type B-1 fire extinguisher(s)
  - * Horn, whistle or bell
  - * Daytime visual distress system
  - * Night time visual distress system
  - * Functioning navigation lights
- VHF radio.
- Boat anchor (line sufficient for a depth of 30 ft).
- Depth pole (8 ft pole, graduated in 1 ft increments) - for determining depth.
- Net anchor (approximately 15 ft of line, with an anchor attached to one end and a carabineer and float attached to the other) - for anchoring the net.

Figure 4. Examples of boats used during Dolphin HERA Project capture-release operations (clockwise from upper left): Catch Boat, Dolphin Processing, Photo-ID, Safety, Sample Processing and Chase Boat

- Clip line (approximately 15 ft of line, with a carabineer attached to one end) - for attaching a boat to the net.
- 2 tri-fold composite mats - for floating and transporting dolphins during deep water sets (Catch Boat and Sample Processing Boat only).

Personnel/Assignments – There are many tasks involved in C-R field operations (Table 2). Tasks are not necessarily mutually exclusive (*i.e.*, an individual may be assigned more than one task or may be required to perform multiple tasks on an *ad hoc* basis). As much as possible, appropriate personnel are identified, assigned tasks and informed of assigned duties during the planning phase. Some tasks require specialized skills (*e.g.*, Catcher, Veterinarian) or benefit from continuity in assigned individual (*e.g.*, Data Manager, Data Collectors). These tasks will be assigned to the same individual, whenever possible, for the duration of the capture. Boat assignments (the boat an individual is designated to be on) can change on a daily basis while others may remain consistent for the duration of the C-R operations. It is the individual's responsibility to know and understand their role and which boat they are assigned to each day of field operations. Any questions pertaining to boat or task assignments should be addressed to the RCA.

Health/Safety Precautions – The health and safety of personnel and dolphins during C-R studies are of paramount importance. As with all wild animals, dolphins are potentially dangerous and can cause traumatic injury, death or harbor zoonotic diseases. Wild animals may have infectious disease and yet have little disease signs, thus, precautions should be taken against transmission of infection regardless of the physical appearance of a captured animal (Cowan *et al.* 2000). Prolonged and frequent exposure to marine mammals was previously described as a significant risk factor associated with injury and illness to humans (Wildlife Health Center 2004). While the amount of exposure time to marine mammals during captures is minimal compared to captive display operations, adherence to safety guidelines, use of protective clothing, common sense and good personal hygiene can substantially reduce risks. The risks can be minimized by

Table 2. List of positions, suggested number, associated tasks and recommended boat assignment

Position	#	Task(s)	Boat Assignment
Capture Manager*	1	• Make final decision on capture attempts • Provide safety and operations oversight	• Chase Boat
Regional Capture Advisor*	1	• Manage regional logistics and advise CM • Decide on daily area(s) of operation • Provide safety and operations oversight	• Chase Boat
Catcher*	1	• Operate Catch Boat • Determine suitability/feasibility of capture attempt • Net operations	• Catch Boat
Captain*	~6	• Operate boat • Function as handler after securing and anchoring boat	• All
Mate	~6	• Assist with boat operations and radio communications • Function as handler after securing and anchoring boat	• All
Veterinarian*	3+	• Monitor status of captured dolphins • Perform (or supervise) sample collection • Perform physical and ultrasound examinations • Affix tags	• Catch Boat • Sample Processing • Chase Boat
Vet Tech	2-3	• Assist vets as required • Sample processing, storage and shipping	• Sample Processing • Dolphin Processing
Data Manager*	1	• Ensure individual dolphin data sheets are completed	• Chase Boat
Lab Manager*	1	• Ensure all samples are processed • Ensure all sample data sheets are completed • Assign persons to laboratory tasks	• Sample Processing
Lab Tech	4-5	• Sample processing • Ensure proper storage of all samples	• Sample Processing
Photographer*	1-2	• Obtain photographic documentation of each dolphin	• Sample Processing • Scout
Freeze Brander*	1	• Freeze brand captured dolphins • Direct handlers in assisting branding process	• Sample Processing
Data Collector*	2	• Collect morphometric data for each dolphin	• Chase Boat
Handler	15-20	• Restrain/support captured dolphins	• Chase Boat

** personnel filling position will remain fixed for the duration of the capture*

incorporating plans for exposure control, training, personal protective equipment and emergency procedures. The website www.vetmed.ucdavis.edu/whc/mmz provides information about possible disease transmission from marine mammals. Precautionary guidelines for conducting C-R studies include some of these established regulations as well adaptations that are specific for conducting dolphin C-R studies as listed in the Safety Protocols and Guidelines (Appendix A) and those noted below.

Researchers should minimize the chances of being injured or bitten and should not put themselves in harm's way. Persons with open wounds, active infections, compromised immune systems or pregnant should not have contact with dolphins. Avoid unnecessary exposure to blood, other body fluids, feces and exhaled air all of which may contain pathogens and parasites. Special care should be taken to avoid needle and scalpel punctures when collecting blood and other biological tissue samples. To reduce the risk of pathogen transmission, directly or indirectly between humans and dolphins, team members should wash their hands with antiseptic foam or dry soap between handling individual dolphins. Use of a bacterial disinfectant agent should be used after direct skin contact with dolphins exhibiting any skin diseases. To minimize transmission of potential infectious agents between dolphins, the Dolphin Processing Boat deck, mats, stretcher and all other sampling equipment should be disinfected after processing each dolphin.

Investigators working with the dolphins should wash field clothes and all materials that come in contact with dolphins, blood or body fluid. Staff should also avoid ingesting food or drink in areas which may have been in contact with dolphins.

Emergency Contingencies – The following contingencies have been established to reduce risks to both personnel and dolphins (also see Appendix A):

- Float plans (detailing all vessels as well as personnel) are filed daily with a responsible, land-based contact person
- Safety Boat (with Emergency Medical personnel) must be on-site during all research activities
- Human health takes precedence over all research activities
- Members of the capture team should be certified in CPR
- Local marine law enforcement, USCG and Fire/Rescue services are informed of planned activities
- Net knives are readily available during net deployment and dolphin capture and disentanglement
- Veterinarians have immediate access to Dolphin Emergency Treatment Kits throughout the capture process
- Dolphin emergencies are handled by on-site veterinarians
- A dolphin that dies during C-R activities will be necropsied by on-site veterinarians and staff (other non-project veterinarians may be included as independent observers/participants)

Capture Protocols

Conditions for conducting captures are detailed in Scientific Research Permit No. 998-1678-00 issued by the National Marine Fisheries Service, Office of Protected Resources to Gregory Bossart. A maximum of 400 individual Atlantic bottlenose dolphins (*Tursiops truncatus*) are permitted to be captured, examined, sampled, tagged and released over a 5-year period in the Indian River Lagoon, FL and Charleston, SC. Dolphins are encircled by a large mesh seine net. Once encircled in shallow water, dolphin handlers and marine mammal veterinarians are deployed around the net circumference (*i.e.*, compass). Once a dolphin strikes the net, it is restrained, stabilized and disentangled by the handlers. It is recommended that there be a minimum of five handlers for each captured dolphin. Capture-release field operations are directed by the Capture Manager (CM) assisted by the Regional Capture Advisor (RCA) as detailed in the Safety Protocols (Appendix A).

Capture Criteria – Several criteria, including location, environmental conditions, age of targeted dolphins and group size and composition must be considered before attempting a capture. Captures should be attempted in calm, shallow water (<2 m deep) over a substrate solid enough for researchers to effectively stand and handle a dolphin. It is important to note that depths are not always uniform at a capture location and dolphin handling procedures vary depending on water depth. Efforts are made to limit capture activities to shallow waters (<2 m deep) and if captures occur in water deeper than 2 m,

the dolphin(s) or net can be moved to shallower water. Calves estimated to be less than 2 years of age are not targeted for capture. Groups of three or more dolphins are not typically targeted for capture unless the group can be effectively split into smaller sub-groups. Finally, attempts are made to minimize the amount of stress to dolphins during the selection process (*i.e.,* limit the amount of harassment of dolphins while their suitability for capture is evaluated).

Locating Dolphins – Dolphins are located either by actively searching for them or by waiting for them to pass by a likely capture location. Dolphins are more oftentimes located actively, by boat-borne personnel surveying the intended area of operations, while the boat(s) are maneuvered through the study site. The second method is more useful when attempting to capture specific dolphins or in locales with limited areas suitable for capture. The method used depends on the research objective(s) and/or the study site. The various boats are directed to search given areas by the CM/RCA; however it is usually best to keep all of the boats reasonably close together in case a capture opportunity presents itself on short notice. In sites with on-going photo-ID studies, local researchers can provide the CM/RCA with advice and guidance in determining areas where capture activities can occur and where dolphins are more likely be found. If no suitable dolphins are located after a period of searching, one or two boats (typically the Photo-ID Boat) may be sent to search areas further from the rest of the boats (while remaining in radio contact).

Photo-Identification of Dolphins – When dolphins are sighted, photo-ID staff first attempt to identify individual dolphins and determine information such as group size, approximate age(s) and sex(es) (if known) of group members. Efforts are also made to identify the presence of young calves or females that might be pregnant (based on sighting history). If the dolphin(s) are deemed suitable for capture, the CM (in Chase 1) approaches the dolphin(s) for final confirmation and approval to set the net. During net deployment, the photo-ID team should attempt to maintain a visual on the dolphins and photo document the net set and subsequent activities.

Net Deployment – Once dolphin(s) are selected and approved for capture, the CM/RCA and/or Catcher will notify the boats to proceed to the selected location. To avoid undue stress to the dolphins, boat captains should avoid approaching targeted dolphins prior to net deployment; typically only the Catch Boat and Chase 1 will closely approach the dolphins while the remaining boats remain nearby. Once a sufficient number of Chase Boats, with an adequate number of dolphin handlers, are on-scene and conditions are safe to attempt a capture, a large (366 m by 7 m) seine net (22 cm stretch mesh with double float and lead lines) is deployed as the Catch Boat accelerates around the target dolphin(s). Following net release, the CM notifies, via VHF radio, all capture personnel of the net release and maneuvers Chase 1 in the opposite direction of the Catch Boat in order to create an acoustic and mechanical barrier to the dolphins until the net is completely closed (Fig. 5). Chase 2 follows astern of the Catch Boat and outside the compass, while crew members watch for net overlays or entangled dolphins. The other Chase Boats should take up positions around the net (or compass) so that the boats are equally spaced around the compass perimeter.

Following closure of the compass the remaining research boats should approach the compass and take up positions as instructed by the CM/RCA. While approaching the net, all personnel should carefully watch for dolphins prematurely striking the net and/or net overlays (where the lead line has twisted over the float line, resulting in the float line being submerged and the lead line lifting off the bottom). It is very important that an accurate count of dolphins in the compass be maintained. Upon nearing the compass perimeter, water depth should be checked manually using depth poles or, in deeper water, via depth sounders. The captain of each Chase Boat should then relay water depth information to the CM.

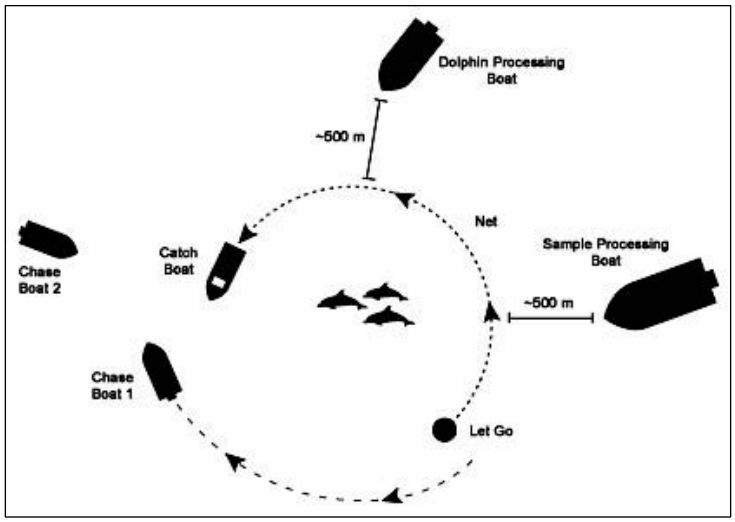

Figure 5. Boat positioning during net deployment (not to scale)

If the compass, or a portion of the compass, has been set in deep water the Catch Boat, can tow the compass towards shallow water. If the set has been made in an area of strong current, boat crews should be prepared to set net anchors to prevent the collapse of the compass. If the compass has been deployed in water depths greater than 2 m or in an area of a challenging environmental condition the dolphin and handlers can be placed on a floating tri-fold composite mat (Ortega Canvas and Sail Repair Awnings, San Diego, CA).

After the net has been secured, experienced dolphin handlers are deployed evenly around the outside of the compass. The Dolphin and Sample Processing Boats should be within 1 km of the set. The Dolphin Processing Boat is maneuvered into shallow water near the compass. Meanwhile, the crew of the Dolphin Processing Boat begins preparations to receive the dolphin(s) and for subsequent sampling procedures. The Sample Processing Boat is rafted along the Dolphin Processing Boat and they are tied securely together and anchored. While the net is being deployed, as dolphins are free-swimming in the compass and during dolphin capture and disentanglement, it is imperative that all personnel adhere to the following safety guidelines (NOTE: see further safety protocols/guidelines in Appendix A):

- Stay calm and quiet (only designated personnel should speak during this process)
- Listen for directions from the boat captain
- Do not dive into the water
- Wear foot protection and shuffle feet when in water to avoid stingrays or other obstacles
- Wear life-vests in deep water sets (all personnel)

Capture and Disentanglement – The behavior of encircled dolphins can vary widely. Dolphins, especially those with no prior C-R or net experience will sometimes strike the net soon after it is set or even while it is being set. Again, it is important that capture personnel remain calm, follow directions and not all attempt to reach the entangled dolphin. If a strike occurs in shallow (<2 m) water, personnel from the nearest Chase Boat will be deployed into the water near the entangled dolphin, on the outside of the compass (Fig. 6). Entangled dolphins should only be approached by multiple handlers; single handlers should not attempt to restrain a dolphin. To avoid becoming entangled, handlers should be cautious around the net, particularly in the vicinity of the dolphin. While the float line might be distant from the handlers the net itself could be much closer because of the actions of the dolphin. Handlers should attempt to first approach and restrain the dolphin in front of the dorsal fin just behind the pectoral flippers and then shift back towards the flukes and toward the head. Subsequent handlers should then fill in along the length of the dolphin's body until it is sufficiently restrained. If possible, handlers should try and arrange themselves so that they are not all positioned on one side of the dolphin. Handlers should minimize the amount of space between themselves and the dolphin to prevent the dolphin from striking them. After buoying the dolphin at the surface and preventing further entanglement, no other steps should be undertaken until instructed by the CM, RCA or an experienced handler.

Figure 6. Buoying and securing entangled dolphin

If the set is in deep water (>2 m) under no circumstance should personnel enter the water until the dolphin has been secured alongside the responding Chase Boat with the dolphin's blowhole at the surface. The dolphin is handled by first maneuvering the Chase Boat to the float line near (not directly at) the entangled dolphin. The crew should then manually pull the boat along the float line until the dolphin is alongside the boat, with crew members supporting the dolphin at the surface by holding the net. At this point, lines should be cleated off to the responding Chase Boat, passed through the mesh of the net entangling the dolphin and hand-tended by the boat crew. This allows handlers to get their hands out of the net and can prevent them from becoming entangled in the net and/or pulled overboard if the dolphin spins or thrashes. Once secured, one or two experienced handlers may then quietly enter the water and approach the dolphin's outside flank to look for net that may be constricting the dolphin and to aid in supporting the dolphin. Handlers should pay close attention to the dolphin's head, particularly the eyes, mouth and blowhole; it may be necessary to cut away constricting sections of the net from these areas. The dolphin can then be transported to shallow water via a floating mat or by towing the net. It is critically important that other boat crews remain aware of any other dolphins within the compass and watch for signs of entanglement (*i.e.*, bobbing cork line, splashing or 'tugs' on the net).

Dolphins don't always strike the net and may remain free-swimming within the compass. In shallow water, boat crews will be directed to enter the water and distribute themselves evenly around the perimeter of the compass. After the handlers are arranged around the perimeter on the outside of the net, the net can be 'pinched' by pulling the opposite sides of the net together either using the Catch Boat or by hand as shown in Fig. 7. The net may also be 'zippered' in order to restrict the amount of area available to the dolphin(s). The CM and Catcher will decide which method is appropriate. These maneuvers, to pinch or zip the net, reduce the size of the compass and ultimately cause the dolphin(s) to become entangled, facilitating capture.

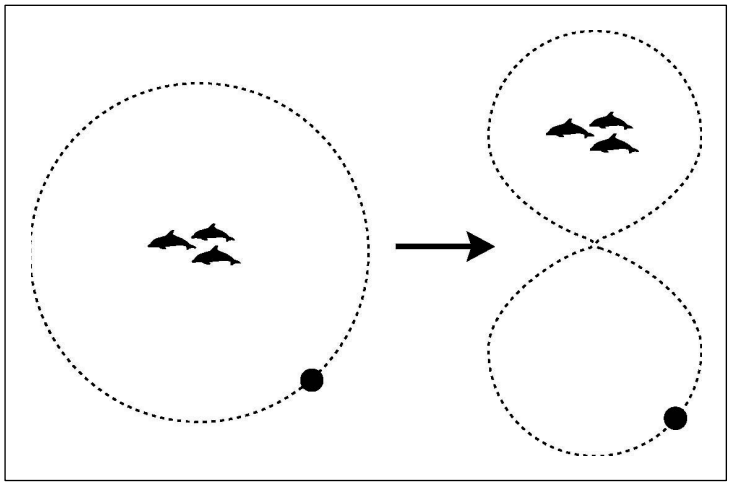

Figure 7. 'Pinching' the net in order to decrease the amount of space available to encircled dolphins

Oftentimes, even within a restricted compass, the dolphin(s) will avoid entanglement. In this case, experienced handlers may enter the compass and try to maneuver the dolphin into the net or attempt to manually restrain a free-swimming dolphin. When a dolphin strikes the net, adjacent handlers should immediately raise the float line and put tension on the portion of the net entangling the dolphin. Holding the float line up may prevent the dolphin from escaping over the net and can limit the amount of net the dolphin has to entangle itself. Handlers should then work their way toward the entangled dolphin by passing the float line hand-over-hand. The first handler to reach the entangled dolphin should attempt to grasp it in front of the dorsal fin just behind the pectoral flippers. Additional handlers should fill in behind the first handler until a sufficient number of people are supporting and restraining the dolphin. Once a dolphin is restrained, handlers should not let go without first communicating with the other handlers. Furthermore, it is important to allow as little space as possible between the handlers and the dolphin in order to prevent possible injury to handlers. Efforts should be made to remove or cut any lines from the inside of the dolphin's mouth. Once a dolphin is sufficiently under control, the handlers should make every effort to keep it comfortable (*i.e.*, don't 'squeeze' too tightly, avoid sensitive areas such as the eyes and blowhole and keep the dolphins exposed surfaces, especially the dorsal fin, wet).

A dolphin can, on rare occasions, be handled and restrained as it swims freely within the compass (an unlikely scenario given the naïve nature of many dolphins). Initial contact with the dolphin is accomplished by one or two experienced handlers with help from additional follow-on handlers (similar to the process described above). This method is not used often but has been effective when employed.

After support and restraint, entangled dolphins must be extricated from the net. When possible, dolphins should be disentangled inside the compass so that if the dolphin

escapes from its' handlers, it will still be within the compass. Disentanglements should be directed by an experienced handler whenever possible. Occasionally net knives may be used to facilitate disentanglement; again, such actions should typically be carried out by an experienced handler.

Processing/Sampling Protocols

All sampling protocols were approved and conducted under National Marine Fisheries Permit No. 998-1678-01 and by the HBOI Institutional Animal Care and Use Committee (IACUC). Once a dolphin is captured and disentangled, it is restrained by three to six handlers, undergoes an initial veterinary assessment and is monitored continuously until

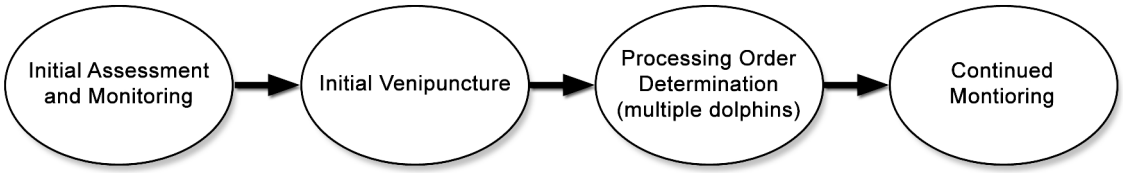

Figure 8. In-water processing performed by veterinarian and dolphin handlers

released (Fig. 8). Each dolphin is assigned a unique identifier (freeze brand ID), which is used to track collected data and samples. Once a dolphin has been stabilized after disentanglement, blood samples are collected. The dolphin is then placed in a stretcher, weighed and lifted onto the Dolphin Processing Boat for a comprehensive health examination and sample collection (Fig. 9). Monitoring continues while the dolphin is

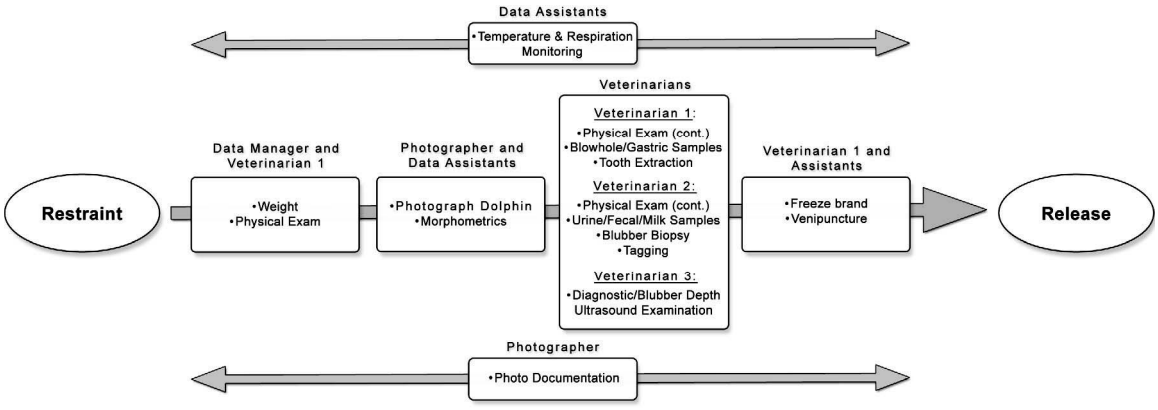

Figure 9. On-boat flowchart

on-board, with heart rate, respirations and skin surface temperature recorded at set intervals. While on-board dolphins are shaded by a Bimini top and wetted continuously with seawater. A comprehensive, standardized evaluation is conducted and includes the following: complete physical examination by an experienced marine mammal veterinarian, diagnostic and blubber depth ultrasound examination, evaluation of body condition, evaluation of skin condition and inventory of skin lesions. Biologic samples collected from each dolphin and include blood, a tooth (if not previously sampled), a

skin/blubber biopsy, biopsies of remarkable lesions, urine, feces, gastric fluid, blowhole cells and milk (if lactating). Swabs are taken from the nasal sac, gastric fluid and feces for culture and cytologic evaluation. The following represents the typical processing sequence: restraint, venipuncture, measurements, physical examination, photographic documentation, blubber and diagnostic ultrasound, microbiologic and cytologic sampling, urine/fecal/milk sampling, tooth extraction, blubber/skin biopsy, freeze branding, tagging, radio-telemetry and release.

Appropriate documentation of all procedures is performed by a designated Data Manager on a series of waterproof field data sheets (Appendix G). The Data Manager is also responsible for ensuring that all relevant data (including samples, measurements, pictures and video) have been collected from each dolphin prior to release. Designated personnel monitor and record respirations and body temperature at three body locations (Appendix Q) using a thermal gun (Raytek, Santa Cruz, CA). The Laboratory Manager is responsible for ensuring that all data associated with analyses conducted on the Sample Processing Boat have been collected.

Restraint – Three to six handlers (the exact number will depend on the size and demeanor of each dolphin) will restrain a dolphin after it has been disentangled from the net (Fig. 10). Appropriate handling and restraint techniques are used to avoid injury to both dolphins and humans. Restraint is minimal and carefully applied. While restrained, an initial assessment is performed and respiration and heart rates monitored. If multiple dolphins are caught, a veterinarian will determine the order in which the dolphins are to be processed.

Figure 10. Restraint

Venipuncture – Blood samples are obtained as soon as possible after the dolphin is disentangled to reduce variation due to time-dependent changes in blood constituents. Blood samples are drawn from the periarterial venous rete in the flukes on the ventral side of the fluke (Fig. 11). A handler lifts the flukes out of the water and the site is prepared aseptically with a surgical scrub (2% chlorhexidine gluconate) and then wiped with a methanol-soaked gauze pad. Once the site has been scrubbed care should be taken to keep the flukes out of the water. Venipuncture is performed by an experienced marine mammal veterinarian using a 19 gauge needle and a 1.9 cm butterfly catheter with a vacutainer attachment (Becton, Dickinson and Co., Franklin Lakes, NJ). Serum is collected in a 10 mL serum separator vacutainer tube

Figure 11. Venipuncture

(Becton, Dickinson and Co., Franklin Lakes, NJ), placed at room temperature for 20-40 min, then centrifuged for 15 min at 1200 rpms and placed in a cryovial (Corning Inc., Acton, MA) on ice. Samples for hematology are collected in a vacutainer tube with ethylenediaminetetraacetic acid (Becton, Dickinson and Co., Franklin Lakes, NJ). Samples are shipped overnight on cold packs to Cornell University Veterinary Diagnostic Laboratory (Ithaca, NY) for complete blood count and a serum chemistry panel. Erythrocyte sedimentation rate (ESR) is measured within 10 min of sample collection using the Sediplast system (Polymedco Inc., Cortlandt Manor, NY). Blood analysis measurements using the critical care I-STAT Unit (Heska Corp., Loveland, CO) are also measured within 10 min of collection when possible. Additional blood samples are collected for contaminant and other analyses.

Measurements – Following blood collection, mature females are checked for pregnancy status via ultrasound (SonoSite 180plus, Bothell, WA). Dolphins in the middle to late second trimester of gestation are typically released immediately, but may receive an abbreviated exam. Dolphins in the third trimester are released immediately with no processing. All other dolphins are placed in a stretcher for weighing and positioning on the Dolphin Processing Boat. A stretcher is brought alongside the dolphin. One of the stretcher poles is then passed beneath the dolphin to the handlers on the opposite side.

Figure 12. Weight (left) and morphometrics (right)

Both poles can then be lifted with the dolphin secure between them. Weights are obtained by hoisting the dolphin out of the water using a boat-mounted davit, block-and-tackle and a four-point harness that clips into eyebolts at each end of the two stretcher poles (Fig. 12). The dolphin is lifted fully from the water and a weight is recorded from a digital scale connected to an in-line load cell (Western Scale Company Limited, Port Coquitlam, BC). The dolphin is then maneuvered over the stern of the Dolphin Processing Boat and lowered onto a padded deck. Morphometric data, such as standard lengths and girths, are obtained using a metric tape measure (as described in Appendix G) while the dolphin is on deck (Fig. 12). Alternatively, measurements may be taken while the dolphin is in the water. Two persons are required to record morphometrics; the same two data collectors should collect all dolphin morphometrics throughout the course of the capture to maintain accuracy and precision between measurements.

Physical Examination – On deck, the dolphin is placed on high-density 5 cm closed-cell foam mats and shaded by a canvas Bimini top. While on deck, each dolphin is supported

by the minimum number of handlers. Handlers support, restrain and position the dolphin while it is being processed and are responsible for keeping the dolphin wet and cool by applying seawater using sponges and/or sprayers. An experienced marine mammal veterinarian conducts complete physical examinations for overall health and external signs of disease on each dolphin (Fig. 13). The physical examination consists of subjective and objective criteria as detailed in the literature (Gage 1990; Geraci and Lounsbury 2005; Bossart *et al.* 2001). It includes a thorough external examination of the integument, eyes, blowhole, genital/mammary region and postnuchal adipose tissue. Breath rate, excursion and blowhole odor are observed. Cardiopulmonary and abdominal auscultation/percussion and abdominal palpation are performed. An oropharyngeal examination is also conducted and includes evaluation of dental health, tongue, gingival, oropharyngeal lymphoid tissue and capillary refill time.

Figure 13. Physical exam

Photographic Documentation – Photo documentation of each individual dolphin visually supports written data pertaining to the dolphin and procedures performed. Photographs are also used to record unusual findings such as skin lesions, injuries, physical abnormalities, human interactions, fisheries interactions and/or predator interactions (*i.e.*, shark bites). Once the initial physical examination is complete, detailed photographic images are taken to document body condition. Three images are taken on both the right and left sides of the dolphin (*e.g.*, head, thorax and peduncle). These images provide a map of any cutaneous abnormalities. Detailed photographs are taken of any unusual features (*i.e.*, skin lesions, scars, etc.) (Fig. 14). A close-up image of the dorsal fin is also taken from each side, using a graduated board (blue or white) as a background. Once the blubber biopsy is removed, detailed photographs are taken of the biopsy site. If any additional biopsies are taken, the same photography protocols are applied.

Figure 14. Photo documentation

Diagnostic and Blubber Depth Ultrasound – Ultrasound is used on mature females for pregnancy evaluation. If a dolphin is pregnant, ultrasound is often used to obtain fetal measurements. Ultrasound examinations are performed on all processed dolphins to measure blubber thickness (Fig. 15). Blubber measurements are taken at seven different sites along the dolphin's body. Testicular length is

Figure 15. Ultrasound examination

measured in male dolphins. In addition to taking measurements, ultrasound is used to detect any abnormalities in the liver, urinary tract, reproductive tract, cardiovascular system and stomach.

Microbiologic and Cytologic Samples – Swab samples are collected from the blowhole, gastric fluid and anus of each dolphin for bacterial and fungal assessments and for cytologic evaluation. A sterile swab is inserted into the blowhole during a breath, gently moved along the wall of the blowhole and removed during the next breath (Fig. 16). Gastric fluid is collected into a sterile conical vial by inserting a well-lubricated soft,

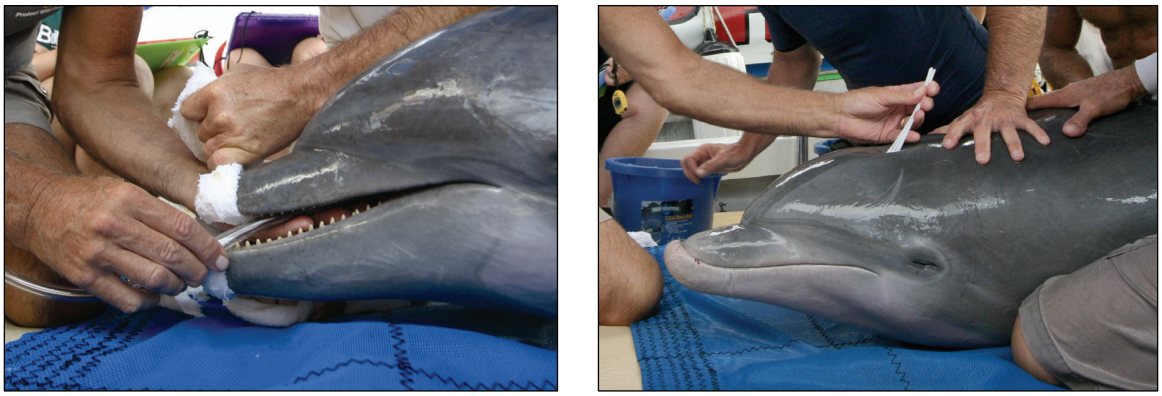

Figure 16. Gastric fluid collection (left) and blowhole cytology (right).

flexible, plastic foal stomach tube past the oropharynx to the first stomach (Fig. 16). A swab is inserted in the gastric fluid and smeared on a histological-grade slide for cytological analysis and gastric pH is recorded. The remainder of the sample is then centrifuged and a slide is prepared with the spun gastric sample. An anal swab is collected by inserting a sterile swab into the anal orifice and gently swabbing the area. Swab samples are placed in transport media in preparation for bacterial culturing and organism identification. Swab samples for cytologic evaluation are thinly smeared on clean histological-grade slides and air dried.

Urine Sampling – Urine samples are collected by urethral catheterization (Fig. 17). Catheterization is performed by an experienced veterinarian and veterinary technician using standard sterile conditions (sterile surgical gloves are worn; catheter and other equipment are sterile). The technician gently retracts the folds of the genital slit to expose the urethral orifice. The genital area is wiped anterior to posterior with clean sterile dry gauze to mechanically remove any debris. A sterile #8 Fr urinary catheter for males (Butler Animal Health Supply, Dublin, OH) or a 10 Fr Self-catheter for females (Best Buy Medical/Healthcare, Santa Clarita, CA) is then lubricated with sterile lubricating gel and inserted into the urethra. Urine is collected into a 50 mL sterile collection tube without additive. Urine sample analyses include routine urine analysis (Chemstrip, Roche Diagnostics, Indianapolis, IN) contaminants, hormones and biomarkers.

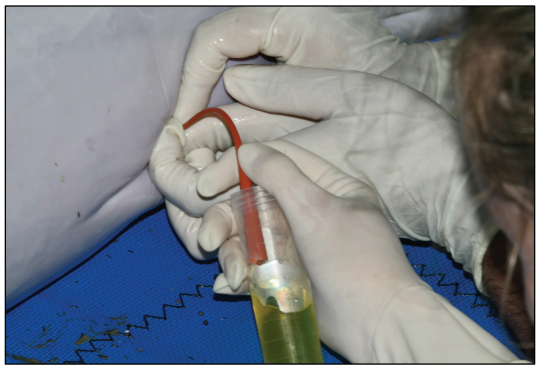

Figure 17. Urine sampling

Fecal Sampling – Fecal samples are collected for cytologic, pathogen and antibiotic resistance evaluation. Samples are collected in a sterile 15 mL conical vial opportunistically as the dolphin voluntarily defecates, or by catheterization (Fig. 18). An experienced marine mammal veterinarian and a qualified veterinary technician perform the procedure under standard sterile conditions (sterile surgical gloves are worn by both; catheter and other equipment are sterile). The technician gently retracts the folds of the anal slit to expose the anal orifice. A sterile 4 x 4 in gauze is swept anterior to posterior. A saline filled 20 cc syringe with a 14 Fr Self-catheter (Best Buy Medical/Healthcare, Santa Clarita, CA) placed on the tip is inserted gently into the anal orifice. The saline is slowly injected as the catheter is advanced. The saline is then withdrawn from the dolphin's body with the fecal material obtained in the syringe.

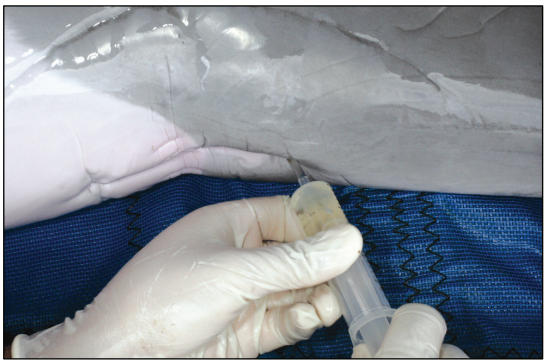

Figure 18. Fecal sample collection

Milk Sampling – Milk samples are collected to assess levels of lipophilic contaminants and to determine composition. All adult females are examined for lactation. The collection site is prepared with an alcohol-soaked gauze pad. Milk is expressed by placing the inverted barrel of a 12 cc syringe, attached by plastic tubing to a 60 cc syringe, over the nipple and applying gentle suction (Fig. 19). Samples of up to 30 mL may be obtained in this manner.

Figure 19. Milk sample collection

Tooth Extraction – A single tooth (left 15th mandibular tooth is recommended) is extracted from every dolphin for age determination, unless a veterinarian determines that the procedure would be detrimental to the dolphin's health. The tissue and root structures surrounding the tooth selected for removal is infiltrated with 3% carbocaine with a standard high-pressure, 30-gauge dental injection gun (Miltex, Dedham, MA) (Ridgway *et al.* 1975). Once the area is anesthetized, the tooth is elevated and extracted under steady and firm traction with the use of a dental elevator and an extractor (Fig. 20). A gelfoam (Butler Animal Health Supply, Dublin, OH) plug is placed in the open sulcus to facilitate clotting and healing. The tooth is placed in a plastic vial and stored at room temperature. Extracted teeth are first sectioned and stained and then age is determined by counting the post-natal dentine layers (Hohn *et al.* 1989).

Figure 20. Tooth extraction

Blubber/Skin Biopsy – A full-thickness skin and blubber wedge biopsy, approximately 5 cm long x 3 cm wide (1 gm), is removed from the left flank of each dolphin at a site located 10 cm posterior and 10 cm ventral to the posterior insertion of the dorsal fin. The site is prepared aseptically with a surgical scrub (2% chlorhexidine gluconate) followed by a methanol-soaked gauze pad. Lidocaine 2% with epinephrine is injected subcutaneously in an inverted 'L' pattern anterior to and above the biopsy site. Once adequate anesthesia is reached (approximately 5 min post-injection), a second surgical scrub of the site is performed. The site then receives a final methanol. A sterile scalpel and forceps is used by an experienced marine mammal veterinarian wearing sterile surgical gloves to excise and remove the blubber biopsy sample (Fig. 21). Following removal, the biopsy site is filled with gauze soaked in ferric subsulfate to promote clotting. The biopsy is divided into epidermis and blubber portions depending upon the analysis to be conducted according to the protocols developed by the National Institute of Standards and Technology (NIST) (Appendix E). Blubber sections are stored in Teflon containers for organic contaminant analysis and in polyethylene containers for perfluorinated chemical analysis. The tissues are then immediately placed in a liquid nitrogen vapor shipper. Blubber is also collected for analysis of fatty acids. Skin is sectioned and portions placed in 20% dimethyl sulfoxide (DMSO) for genetic analyses, frozen for trace metal analysis and for biomarker analyses. Skin samples are also collected for stable isotope analysis (carbon, nitrogen, sulfur). Other samples of blubber and skin are placed in histology cassettes and fixed in 10% neutral buffered formalin for biomarker analysis and histological evaluation. Smaller incisional wedge biopsies are also taken aseptically by the field veterinary staff from all cutaneous lesions considered potentially significant. Grossly observed skin lesions are placed in 10% buffered formalin and/or frozen in a liquid nitrogen vapor shipper. Portions of cutaneous biopsies are also stored in sterile conical vials without fixation media for submission as microbiologic cultures.

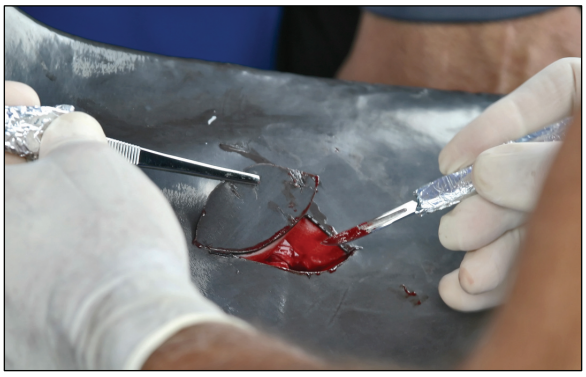

Figure 21. Blubber biopsy

Freeze Branding – Captured dolphins were freeze branded on both sides of the dorsal fin with a unique, three digit code (Appendix E). Freeze brands were in turn used to identify individuals during photo-ID research and track collected data and samples. Females were branded with odd numbers, males with even numbers. The first digit of the freeze brand indicates the study area where a dolphin was captured (*e.g.*, freeze brands of dolphins captured near CHS all begin with the number '8'). Each metallic digit, approximately 2 in x 1 in, was applied to dried skin for 20 sec after being supercooled in liquid nitrogen (Fig. 22). After removing the branding iron, a photograph was quickly taken of the fresh

Figure 22. Freeze branding

brand, the dorsal fin was submerged in the surrounding water and the branding site was vigorously rubbed to return the tissue to normal body temperature. Freeze brands can provide an unambiguous, long-term means of positive identification, of both free-ranging and stranded dolphins.

Tagging – Rototags (Nasco, Fort Atkinson, WI) were used to temporarily mark and identify captured dolphins (Fig. 23). These plastic tags were clipped through the trailing

Figure 23. Rototags (left) and radio-tag (right).

edge of a dolphin's dorsal fin (Appendix F). The large, brightly colored tags enabled the identification, and avoidance, of previously captured dolphins during on-going C-R operations. Tags were generally shed within a year of tagging, typically leaving a small notch or hole which were in turn used as identifying marks in ongoing photo-ID studies (*e.g.*, Scott *et al.* 1990).

Radio-Telemetry – The use of VHF radio transmitters on some of the captured dolphins enabled researchers to collect data on short-term movements, document association patterns and assess post-capture behavior and overall health (*e.g.*, healing of biopsy wounds and freeze brands). Small (<14 g) radio-tags (Advanced Telemetry Systems, Isanti, MN) were affixed to the trailing edge of dolphin's dorsal fins with either rototags or bullet tags (Trac-Pac, Ft. Walton Beach, FL) (Fig. 23). Tags were typically shed within six months of tagging, usually leaving a small notch or hole in the trailing edge.

Release – The dolphin is returned to the water by passing the stretcher off to a receiving team in the water at the stern of the Dolphin Processing Boat. Independent dolphins can be released singly or together upon completion of sampling (Fig. 24). Females with dependent calves (<2 years) are released together. Dolphins are removed from the stretcher, restrained by handlers and positioned in the direction of deeper water, away from boats and the net. Handlers are positioned on the same side of the dolphin (to facilitate a smooth and safe release) and simultaneously release and push the dolphin away from them. Extra care should be taken when releasing dolphins that have been instrumented with a radio-tag or other device in order to avoid damaging or dislodging the attachment.

Figure 24. Release

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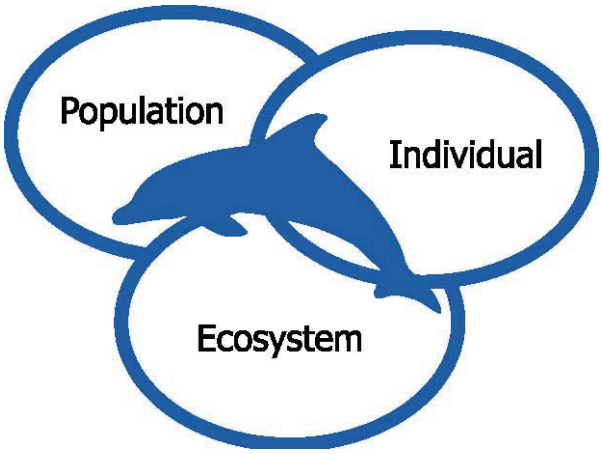
APPENDIX A

Safety Standard Operating Protocols for the Dolphin Health and Risk Assessment (HERA) Project

Dolphin Capture-Release Field Research Activities in the Indian River Lagoon, FL and Charleston, SC



Harbor Branch Oceanographic Institution, Ft. Pierce, FL and
NOAA/NOS/Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC

<u>TO ALL PARTICIPANTS</u>: Please read and acknowledge your full acceptance and understanding of this important document by signing the final page as indicated.

Introduction

The Dolphin HERA Project is a collaborative research effort between Harbor Branch Oceanographic Institution (HBOI) and the National Ocean Service (NOS)/Center for Coastal Environmental Health and Bimolecular Research (CCEHBR). The purpose of this scientific study is to evaluate the health of dolphins at two southeastern sites: the Indian River Lagoon, FL (IRL) and Charleston, SC (CHS). Research activities are authorized under Scientific Research Permit No. 998-1678-00 issued by the National Marine Fisheries Service (NMFS) to Gregory Bossart, V.M.D., Ph.D., Director of HBOI's Division of Marine Mammal Research and Conservation. As the Principal Investigator, the permit holder must authorize any and all final decisions regarding these operations, subsequent research activities, dissemination of data, use of photographic images and publication of data. In the absence of the permit holder, the Capture Manager assumes overall C-R operational responsibility and may delegate on-water operational responsibility to the Regional Capture Advisor or one of the co-investigators as deemed necessary.

This document has been prepared for Dolphin HERA Project participants and provides safety protocols and rationale for conducting capture-release (C-R) operations in association with the Dolphin HERA Project in the IRL and CHS. This research is conducted in a potentially hazardous marine environment, and involves the collection, sampling and handling of wild bottlenose dolphins (*Tursiops truncatus*) in variable open water environments. Daily operations require the coordinated use of a minimum of 7 research vessels, deployment and recovery of a large seine net, the handling, restraint and sampling of large marine mammals and the collaborative efforts of 30 to 60 specialists. Safety is a project priority and becomes the shared responsibility of each Dolphin HERA Project participant. With human safety being of paramount concern, the following information provides specific safety protocols and operating guidelines that will help ensure the safety of all Dolphin HERA Project participants. Prior to commencement of research activities, you must advise both the Regional Capture Advisor and Medical Safety Officer if you:

- Have any pre-existing medical condition(s) or physical limitations
- Depend on prescribed medication and/or have special needs or circumstance
- Have a medical allergy or any adverse reaction to bee and/or jellyfish stings
- Are pregnant (due to physical demands coupled with adverse environmental conditions, remote areas of operation and the potential for injury and/or disease transmissions, it is recommended that pregnant women not participate at any level of Dolphin HERA Project C-R activities)
- Have a health risk that could endanger yourself or may limit others
- Have active certification in first aid and/or Cardiopulmonary Resuscitation (CPR) (identify in advance to both the Regional Capture Advisor and Medical Safety Officer by signing the Safety/Medical Staff list distributed via e-mail)

The following acronyms are used throughout this document to identify key personnel: Principal Investigator (PI), Capture Manager (CM), Regional Capture Advisor (RCA), Medical Safety Officer (MSO), Emergency Medical Technician (EMT) and Ships Mechanic (SM).

Roles of Key Personnel

Capture Manager (CM) – The CM oversees all on-water activities and manages resources needed to conduct and maintain safe and efficient operations. The CM operates aboard Chase 1 and, in close cooperation with photo-ID staff and the captain of the Catch Boat, makes the final decision regarding 'net sets'. Whenever possible, the CM will remain aboard Chase 1 to direct and deploy necessary resources and people. When dolphins are encircled in the compass, the CM will further deploy and direct personnel to safely position themselves along the net float line, manipulate the compass and secure and restrain individual dolphins. Following deployment of the net, during the time that dolphins are being secured and restrained and up until the point that they are released, the CM serves as the 'control point'.

Regional Capture Advisor (RCA) – The RCA works closely with the CM in directing capture efforts and oversees dolphin care. Once dolphins are on the Dolphin Processing Boat, operations are under the direct supervision of the senior veterinarian. The RCA coordinates and manages daily operations, equipment, boats and all marine assets.

The RCA is also the central point of communications. All vessels are equipped with VHF radios, which serve as the primary source of field communication. VHF marine radios (on a specifically assigned 'working' channel) are utilized for ship-to-ship and ship-to-shore communications. Boat captains should limit VHF radio conversations to allow for necessary communications between the CM and the Catcher. To avoid confusion, distractions and/or interruptions, only authorized cell phones will be allowed on the water. Participants are encouraged to either leave personal cell phones at the dock, or turn them off and store them in a dry bag.

Medical Safety Officers (MSO)/Emergency Medical Technician (EMT) – During capture activities, both the MSO and EMTs must be on-site. A dedicated Safety Boat shadows and supports the research team. The MSO and EMT enhance overall safety and provide rapid response to any human injury. The MSO and EMT personnel are under the direction of the CM/RCA. Both the MSO/EMT remain in close proximity when the net is deployed, and visually survey in-water operations as experienced marine mammal handlers respond to manage dolphins in and around the net.

In order to best observe in-water activities, the MSO and EMT maintain active surveillance of the compass from elevated positions aboard the Safety Boat. This allows Safety Team members to visually monitor people in the water and quickly alert the CM/RCA of any potential problems.

In the event of an emergency, the Safety Boat is the first response marine unit and contains all essential emergency medical supplies and diving equipment. On-board both

the Safety Boat and Chase 1 are 'First Responders Directories'. These waterproof documents provide a complete listing of all regional emergency resources with contact names/numbers for all fire safety and health care responders and facilities, regional access roads and areas, marinas, boat ramps, safe harbors and protected areas. Additionally, all boats are equipped with a primary first aid kit.

In the event of a human related accident, the CM and RCA work to support the MSO with all available resources while determining the best means to expedite medical transport to the nearest health care facility. The MSO and EMT staff directs all necessary medical efforts to provide for a maximum safe response and immediate assessment of the injury. Injured personnel will first be stabilized on-site and prepared for transport to the nearest shore-based ground transportation location and hospital. Provisions for alternate medical response providers, emergency contacts list and alternate pick-up locations (boat ramps, docks, access areas) have been identified for all areas of operation. In the event an accident occurs in a remote area, response times can be reduced by exploiting all available regional resources. If required, additional medical assistance can be provided from staff veterinarians and other team members who have certification in first aid and/or cardio-pulmonary resuscitation (CPR).

Note: In the event of a life threatening injury, the USCG is notified immediately, via VHF Channel 16, and a request for air transport is made.

Ships Mechanic (SM) and Boat Maintenance – All vessels are USCG certified and are fully serviced and inspected before C-R operations begin. In order to ensure operations are not interrupted, a dedicated SM is assigned to maintain spare parts and be available to support the RCA and all boat captains to maintain smooth, safe and efficient operations. Boat captains will immediately inform both RCA and SM of any operational difficulties or possible boat-related problems. The SM also maintains spare boat keys, a tool kit and can provide access to additional external resources if necessary. The SM also provides each boat with specific tools needed for any unexpected minor repairs and/or maintenance that might occur on-water. Being able to make repairs at sea greatly enhances operational integrity and prevents delays.

Safety Equipment

In order to maximize and enhance safety in the working environment, each vessel is equipped with USCG approved safety equipment, fire extinguishers and first aid kits. Additional safety equipment includes:

- Oxygen (DAN O_2 Kit)
- Automatic Electronic Defibrillation (AED) units (2)
- Floating spine board and neck/spine collar with aerial lift slings
- Hand-held air tanks ('pony' bottles) (30 breaths)
- Scuba air tank (80 cu in) with octopus regulator
- VHF radios/cell phones for ship-to-ship and ship-to-shore communications
- Secondary paramedic safety bag
- Portable eye/skin wash unit/protective eye wear

- Betadine scrub kits, disinfectant scrubs and rubber gloves (as precautionary measures against potential zoonoses)

All safety equipment will be centralized with the MSO on-board the assigned Safety Boat. The primary Safety Boat is designated by the RCA and managed by the MSO.

Safety/Response Protocols

The following protocols are essential components of the safety plan for the Dolphin HERA Project C-R operations. In the unlikely event someone is injured, or if another unexpected situation should arise, please listen to assigned staff members and cooperate fully. To ensure a safe working environment and the ability of the research team to respond, recover and resume operations, it is essential that all participants attend orientation meetings and become familiar with all safety protocols in advance of the commencement of fieldwork.

Serious Injury or Medical Emergency – In the event of serious injury or medical emergency:

1. Immediately notify the MSO/CM/RCA who will direct medical response efforts.
2. Until the MSO or EMT arrives, and without risk to yourself, work to stabilize and support the injured person being careful to limit any movement of the neck and spine.
3. Help secure area (Notify 911 via cell phone and USCG via VHF Channel 16), alert all other research vessels (by secure VHF channel) and consult with the First Responders Directory to direct shore-based emergency response units to the nearest point of pick-up.
4. Make ready a designated first aid platform (Safety Boat) with all necessary medical treatment kits (note: assigned Safety Boat, should be fast, stable (soft and dry ride) and large enough to accommodate safety backboard, equipment and support personnel).
5. Begin a safe release of all restrained dolphins as directed by the PI, CM and/or RCA.
6. Cease all activities and await instruction from the Permit Holder and/or assigned PI.
7. The MSO will take charge and implement the following measures:
   - Check for pulse (CPR/AED - Auto Defibrillator kit - **Yellow** Pelican Case)
   - Stop the bleeding (first aid kit - **Orange** Pelican Case)
   - Clear the airway, support with O_2 (DAN O_2 kit - **Green** Pelican Case)
   - Treat for shock (keep warm, comfortable and shaded)
   - Splint all possible fractures (air inflated splints)
   - Prepare for transport (RCA, MSO and boat captains)
   - Evacuate injured victim(s)
   - File timely accident report and attend debriefing meetings
8. Following safe evacuation of any injured persons(s), all capture-related activities will cease and all boats will return to the base of operations (dock) or, as directed by the PI or CM. Once ashore, all vessels need to be secured and any evidence safeguarded and photographed before boats are cleaned and/or refueled. A mandatory safety investigation (de-briefing) report and/or police and insurance claim report(s) will

commence. All potential witnesses must remain available for interviews. Appropriate follow up will take place with the injured party and/or their representatives.

Boat Fire – Boat fires are serious and immediate threats to human safety and can quickly spread. Exposed vessels are subject to winds, fuels and other marine flammables (wood, fiberglass, etc.) that can ignite without warning and spread rapidly. Fuel and oil can quickly spread across the surface of the water and go unnoticed until ignited. Therefore, any fire should be quickly contained and at the same time, all vessels should protect passengers by working to isolate their vessels upwind from the source of the fire. Only if called upon by the CM should a vessel attempt to recover passengers from the water or assist with efforts to contain the flames. In the event of a boat fire, the boat captain should take the following steps:

1. Remain calm
2. Verify that all personnel are wearing Personal Floatation Devices (PFDs)
3. Radio Chase 1 with a description of the emergency and accurate location (GPS coordinates)
4. The CM will then relay emergency notification to USCG (VHF Channel 16)
5. Work to contain and control flames
6. Evacuate or abandon vessel if necessary (as instructed by boat captain)
7. The CM will direct rescue efforts and instruct other vessels
8. Evaluate and treat any injured personnel aboard the Safety Boat

If the burning vessel is rafted to other boats, all lines should be cast or cut, in order to isolate the fire from other property and help prevent it from spreading. All additional resources and fire extinguishers should be utilized to contain the fire.

If unable to control flames, and if possible, safely tow the burning craft into an open area down-wind from other vessels and property and secure with the boat anchor. If such attempts are unsuccessful, or deemed too dangerous, then the vessel should be abandoned and allowed to drift and burn in an open area.

Inclement Weather/Lightning – Atmospheric phenomenon are unpredictable and represent extreme threats on open water. Fast moving storm fronts and microbursts may bring strong winds, increased wave height, heavy (blinding) rain and lightning. During summer months, afternoon storm cells and associated lightning (ground strikes) are prevalent throughout the southeastern coastal and gulf regions of the United States. During such weather events, VHF weather monitoring, radar and additional resources are utilized to help avoid or limit exposure to the elements.

If approaching bad weather threatens the area in which C-R operations are taking place, the decision of the CM/RCA will prevail. As directed, dolphins need to be safely released and the net secured aboard the Catch Boat. Any dolphins on the Dolphin Processing Boat need to be assessed by the senior veterinarian and then safely released by the in-water receiving team. All staff will return to their assigned boats and secure vessels for foul weather (windscreens zipped down, running lights on, antennas down, don rain gear, etc.). The RCA and CM will coordinate and direct all boats to travel to the closest safe harbor until conditions allow operations to resume. (Note: slower boats should be

lightened and depart first). Alternate ground transportation may be utilized if boats are unable to safely return to base of operation (dock) for an extended period of time.

Sinking Vessel – If for any reason a boat begins to take on excess water and/or is in danger of capsizing, act immediately; verify all personnel are wearing PFDs, issue flare signaling kit to crew, attempt to bail water or stop source of incoming water and issue a 'Mayday' call for assistance on the working channel and/or on VHF Channel 16). Provide vessel name, location, number of passengers and status. Radio and/or signal any nearby vessels. Abandon boat only as a last resort. The boat captain may make an attempt to slowly ground the boat in shallow water. Remain alert, stay calm and follow instructions of the boat captain. (Note: If abandoned vessel remains floating, stay with vessel as this will make it easier to locate passengers).

Vessel Aground – Running aground in the shallow waters of the Indian River Lagoon or other shallow coastal regions is always a possibility. Boat captains and crew should first familiarize themselves with regional navigation charts that are provided for each boat. Boat captains should wear polarized sunglasses that enhance the distinct color changes of water, which indicate changes in water depth. If vessel begins to ground, notify crew to prepare and immediately bring the boat throttle to neutral and simultaneously kill and tilt up the outboard motor. Check crew for injuries and inspect vessel for damage. If necessary, notify the CM on the working channel. If possible, utilize depth pole to push boat to deeper water; if necessary, safely evacuate passengers up-wind from boat and attempt to refloat vessel to deeper water by lifting/pushing in unison.

Operation and Safety Guidelines for Research Vessels

General Boat Operations/Boat Captain Responsibilities – Boat captains are responsible for the safety of their crew and for the safe operation, maintenance, docking and cleanliness of their respective vessels. Boat captains should familiarize themselves with the operational characteristics of their boat and safety equipment. If any boat captain is unfamiliar with the region, they should consult navigational charts, exercise great caution and reduce speed in shallow areas. Use depth poles to confirm depth readings of on-board electronic sensors. Be aware of all posted ICW markers, channel markers and speed (manatee) zones. If you are unsure of depth, trim the outboard motor up and slowly make your way to deeper water before resuming on-plane speed. Be careful not to disturb sea grasses or marine environments (pole boat to deeper water if necessary). EACH boat captain is responsible for checking to make sure a sufficient number of PFDs are on-board AT ALL TIMES. Following daily operations, boat captains should refuel, clean and secure their vessel in the assigned dock slip each night. Report any problems or missing equipment to the RCA immediately. At the end of C-R operations, the RCA will direct efforts of each boat captain to help secure, clean, inventory and manage all respective marine assets.

Personal Flotation Devices (PFDs) – To fully comply with NOAA regulations, Boater Safety Programs and Dolphin HERA Project Safety Policies, **All** Dolphin HERA Project participants are required to wear PFDs while aboard all research vessels while underway. Use of PFDs for in-water personnel is mandatory unless otherwise directed or approved by a supervisor or in instances where a PFD may interfere with the safe handling of a dolphin. PFD vests (Fig. 1) increase visibility and aid in locating people from a distance. PFDs also provide flotation and added support while managing dolphins in deep water. Additionally, PFDs give added protection against possible impact from the dolphin's rostrum or flukes. C-R staff must be aware that no PFD will fully support a person who might become entangled in the net. Each member of the Dolphin HERA Project C-R operations will be assigned an individual PFD and is responsible for it during the course of fieldwork. At the end of C-R operations, all PFDs should be cleaned and rinsed with fresh water before being returned to the RCA. Two types of PFDs are utilized on Dolphin HERA Project C-R operations: Fixed (Zippered) PFDs for C-R staff are configured with minimal straps and front buckles to avoid entanglement in the net. For the majority of staff and crew aboard the research boats, a lightweight auto-inflate (Mustang-Style) PFD is used. The latter PFD is lightweight, cool and allows for a greater range of motion while deflated. Upon entering the water, this style PFD automatically inflates (manual inflation is optional).

Figure 1. Personal flotation device (PFD)

Visibility – During all times that you are in the water, remain visible (in the 'line of sight') of boat captains. Be careful not to obstruct the boat captain's view while underway and do not hesitate to alert the boat captain to any water hazards or wildlife outside of their field of vision.

Boat Propellers – In or out of the water; sharp multi-bladed boat propellers can cause serious injuries, mutilation, dismemberment or death. Boat captains will be responsible for operating boats in a safe manner and remain aware of any swimmers and/or dolphins in their operating zone. The command "STOP" or "NEUTRAL" will be called to immediately bring the boat motor to neutral and/or engage the kill switch. Before restarting a boat engine and placing it in gear, each boat captain must look and call "Clear" to make certain that no personnel are near the propeller. NEVER use the propeller or lower unit of an outboard motor to climb aboard any vessel. Remember, on shore, exposed boat propellers can be equally dangerous. Give a wide 'walk around' to trailered boats in order to prevent accidental contact injury.

Hands, Arms, Legs and Fingers – The Dolphin Processing and Sample Processing Boats are rafted alongside each other while sampling operations are underway. All boats utilize heavy-duty protective rubber bumpers (placed between boats) to avoid damage to both people and boats. Do NOT place your hands, fingers, arms or legs alongside the boat (gunnels) when near potential hazards such as other boats, docks and pilings. Such accidents are often unexpected and are very unforgiving.

Boat Wakes – Be alert to other boat traffic and alert others as necessary. Other vessels operating in/or near the C-R site can produce large wakes, especially along shorelines. Boat captains should remain alert and attempt to request, via VHF Channel 16 or by visual means (flag), a 'slow pass' from approaching vessels. Even small wakes can cause rafted boats to slam against each other. Prevent serious injuries; NEVER place yourself or your limbs between any two vessels.

Boat Anchors, Lines and Bumpers – Each boat is equipped with a primary anchor and a net anchor (which can serve as a second boat anchor), as well as dock lines and bumpers. In-water staff should be aware that outstretched (partially submerged) lines and ground anchors present an unseen hazard. Please note the position of extended anchor lines when working in the water and exercise caution when deploying and retrieving anchors. Safely stow anchors and all lines before getting underway.

Boat Towing (On-Water) – The RCA will oversee boat towing and coordinate with boat captains and the SM. A balanced towing harness is essential to conduct a safe, efficient tow. The boat captain of the towed vessel should first place engine in neutral and center the steering. Take any unessential equipment and people off disabled craft. Stay well away from the towline trajectory and be aware that heavy boat lines (and metal boat cleats) can unexpectedly break and become high speed flying projectiles. Boat should be towed to nearest point of repair or extraction (boat trailer ramp). If necessary, local services of Sea Tow or Boat U.S. can be utilized by the RCA.

Boat Towing (On Land) – Boat towing will be done only under the supervision of the RCA or other responsible party. Check all trailer lights, tire pressure, trailer hitch and lock-pin connections. The use of stern tie down straps is mandatory. While trailering over long distances, it is advisable to periodically inspect and lube all trailer wheel bearings and to take a spare tire, jack and replacement light bulbs.

Docks and Boat Ramps – Use caution in/around marina area, boat ramps and dock areas. Use caution around slippery and uneven surfaces. Use added caution when boarding or disembarking from any watercraft or when transferring equipment and personal gear between boats. Loose items or items of value should be secured before entering the dock area. Wear anti-skid (white) deck soles or booties on boats. Boat ramps (and boat decks) are notoriously slippery near high tide lines.

Boat Launch/Recovery – Launching and recovering boats will be done only under the supervision of the RCA or other responsible party. Use caution. Be respectful of others. Keep off active ramp when staging or breaking down equipment (boats and trailers). Prior to launching any boat, make sure mooring lines are attached fore and aft and that boat bumpers are in place and safely secured to prevent damage.

Electrical Shock – Use caution aboard the Dolphin Processing and Sample Processing Boats where 100/VAC is utilized to power hoists and sample processing equipment (*e.g.*, centrifuges). In areas where electric power and electronics are in use, remember to keep dry and avoid splashing any power converters, generators and/or power strips. NEVER touch any electrical cord unless you are completely dry and have been directed to do so

by the boat captain. Any plug-in electrical connections (power invertors) need to be insulated to prevent shortage, electrocution and/or damage to equipment. Use of GFI circuits is preferred. Inspect all connections daily.

Anthropogenic Noise – Boat captains should always take care when operating boats in around the net and/or where dolphins are being restrained. Whenever possible turn engines off, do not drop or throw anchors and avoid unnecessary noise that might agitate or alarm dolphins. Remind and caution crew against jumping, splashing or banging equipment on the boat hull. Be careful, do not jump in the water when dolphins are being restrained, instead, use the boat ladder. Do not allow metal stretcher poles to touch and bang underwater near dolphins and never stand directly in front of any dolphin while it is being restrained.

Caustic Chemicals – Certain operations on both the Dolphin and Sample Processing Boats involve the handling of hazardous materials and liquids. It is essential that lab personnel and others wear protective clothing (OSHA-approved gloves, aprons and/or eyewear) and take precautionary measure to limit risks. **In the event of skin or eye contact with a caustic liquid or material, immediately have the nearest boat captain alert the MSO to aid in your use of the portable eye/skin wash unit aboard the Sample Processing Boat. If needed, additional fresh water may be obtained from water coolers aboard each boat.**

Liquid Nitrogen (LN_2) – LN_2 is used to chill the branding irons used to freeze brand dolphins. Skin exposure to LN_2 can result in serious injury and/or severe burns. **Therefore, all LN_2 containers need to be well marked and securely stored to prevent accidental spillage.** All personnel involved in the handling of LN_2 should take every necessary precaution and utilize approved gloves and eyewear to limit accidental exposure to themselves or to others. At times when LN_2 is in use, any/all non-essential personnel should stay clear, especially when LN_2 is being disposed of or transferred between containers. Chilled branding irons remain at sub-zero temperatures for some time after use and should be handled with caution until return to ambient temperatures. In the event of contact with LN_2, immediately and continually douse with water, while the MSO is notified.

Exposure to the Elements – Special caution needs to be taken when working for extended periods of time in the hot sun, cold water and/or when exposed to wind, rain or any combination of elements. Heat stroke is of particular concern. Initial symptoms of heat stroke are mild and are sometimes difficult to recognize; symptoms may include slight dizziness or nausea. More serious symptoms such as blurred vision, slurred speech and inability to stand or walk can immediately follow, including loss of consciousness, or stroke, which can lead to death. Therefore, personnel experiencing such initial symptoms should immediately notify their assigned 'buddy' and seek proper treatment from the MSO. Until aid arrives, seek shade, rehydrate with small amounts of drinking water and stay calm.

Emergency Breathing Equipment – In the unlikely event of an accident or net entanglement that prevents a person from rising to the surface, all efforts will be made to

immediately provide an emergency air source to aid the victim. Both hand-held and scuba (with an octopus regulator) air tanks are available aboard the Safety Boat. A second hand-held tank is kept aboard Chase 1. The scuba equipment can also be used to assist with boat repairs or damage assessment of vessels.

Capture Net – 'RESPECT THE NET'

The net is the primary responsibility of the Catcher. The Catcher is responsible for the deployment and recovery of the net and works closely with the CM and RCA.

The capture net is a long (366 m), deep (7 m) 22 cm stretch mesh seine net with double float and double lead. The net is made of heavy-duty materials and has been treated to be weather and salt resistant. It is abrasive and can cut, entangle, dismember or drown dolphins and/or people. Net materials are sensitive to prolonged exposure to the sun and seawater. Rodent infestation and 'net rot' are also prime considerations for long-term care. Please follow instructions on how to care for the net (rinse, dry, load and unload into net box). Loading the net on the Catch Boat is done ONLY under the supervision of the Catcher. This will minimize the potential for bad net sets, net overlays (*e.g.,* lead lines lifted and stuck over the float line) and other limiting factors in the swift and safe deployment of the net.

Dolphin Sightings and Boat Operations – Outside of capture, sampling and processing activities, the RCA is responsible for the safety and operational integrity of the research vessels. Each boat captain is responsible for their assigned boat and crew. All support boats work to assist with initial sightings and/or focal follows if requested by the CM/RCA. No boat should approach dolphins unless given authorization by the CM/RCA because all such 'takes' must be recorded on assigned data sheets (as per permit requirements). If a boat crew happens to sight a dolphin group, that information should be relayed to the CM/RCA.

In order to help quickly identify dolphins and estimate group size, photo-ID staff members, familiar with regional dolphins, are placed aboard the Catch Boat (or Chase 1). Information is provided to the CM before the 'confirmed' call to set the net is made by the CM. All efforts will be made to limit C-R activities to waters 2 m deep or less. This helps ensure the safety of both the dolphins and members of the C-R team.

Setting the Net – Environmental factors, water depth, currents, tidal conditions and proximity of other Chase Boats must all be considered and assessed BEFORE authorization to 'set the net' is given. The CM will be the only one to give the go ahead to the Catcher. The Catcher determines precisely when to execute this instruction.

Once the 'net ball' is thrown from the Catch Boat, the CM will radio "*Net Out*" over the working channel to notify all C-R personnel that a capture attempt is in progress. Any persons on-board the Catch Boat should stand clear and hold fast when the net is being deployed from the stern of the Catch Boat. Any contact with the fast deploying net can result in entanglement and cause severe injury or dismemberment. If entangled in the net,

do not attempt to stop or hold fast. If you feel the net tightening on any part of your body, immediately call to the Catcher to "Stop".

Once the net is deployed, it is referred to as a compass and direction is then identified or referred to by points (north, south, east and west). The CM/RCA will manage the compass, direct Chase Boats to specific areas around the compass and oversee efforts to safely subdue, secure and restrain dolphins. Be aware of other (potentially harmful) animals that might be in the net (*i.e.,* sharks, rays, turtles, manatees, alligators and large fish). All such incidental fisheries interactions must be documented and recorded. Immediately, call out to the CM or RCA if you see any such interaction. Never attempt to manage an entanglement of any kind without the assistance and support of team members.

Entering the Water – **Do not enter the water unless you are designated as an experienced marine mammal handler and have been instructed to do so.** Every effort will be made to set in shallow water. If a set occurs in water greater than 2 m, the CM will direct experienced personnel to respond as described in the C-R SOP. All in-water staff will be paired with an assigned 'buddy'. Everyone will use the buddy system – know where your buddy is at all times.

Handling the Net – **DO NOT approach the net or react to a dolphin entanglement by yourself and never attempt to grab a dolphin by yourself or without the direction and assistance of an experienced marine mammal handler!** One person cannot effectively restrain a wild dolphin and will put themselves (and others) at risk if they attempt to do so. Wait until there are additional experienced handlers to assist in the restraint of any dolphin. If you or a dolphin becomes entangled, if the net is caught on the bottom, or if you are unable to keep your head (or the dolphin's blowhole) safely above water, call loudly for help and relax until help arrives. Every effort will be made to immediately aid and float any compromised swimmer(s). A Chase Boat will deploy to any area where the float line is being pulled under or is bobbing at the surface.

Never lock your fingers in or around the net mesh or float line or allow yourself to become entangled in the net. If you feel the net becoming tight on any part of your body LET GO and WITHDRAW IMMEDIATELY! Stay off the lead line, especially while the net is under tow or is being pulled into the Catch Boat or onto the beach. When responding to an entanglement during a deep water set, a boat hook can be used to grab and pull the float line to the responding Chase Boat. A net clip line can then be quickly secured to the float line and fastened to the Chase Boat (cleat). This allows a dolphin to be safely managed from the gunnels of the boat, rather than in deep water by staff. At a point where the dolphin(s) are secured, assigned staff can enter the water to better assess, aid in handling and transition the dolphin from the side of the Chase Boat to a floating mat. Once on the mat, the dolphin can be disentangled from the net and towed to shallow (<2 m) water.

Dolphins in the Net – Once the net is set, listen to the instructions of the CM. The CM will direct Chase Boats to strategically deploy and dispatch experienced personnel around the compass. A Chase Boat will immediately go to any area where a dolphin hits the net. Preferably, dolphins will remain in the center of the compass, while Chase Boat crews

deploy around the compass. If necessary, in-water handlers can 'splash' the float line when dolphins approach their position. This may help to discourage a dolphin from charging or probing the net while the net is being drawn down or before sufficient personnel are in place. Be aware of any dolphins in the compass and note their number and location. Depending on circumstances, conditions and number of dolphins, attempts may be made by the CM to maneuver the net so as to split, isolate or crowd the dolphin(s) to a selected section of the net. When a dolphin hits the net, efforts are made to quickly (and calmly) secure its head/pectoral area first, by grabbing behind the pectoral flippers and in front of the dorsal fin; additional handlers should then fill in toward the flukes and toward the head until the dolphin is sufficiently restrained. Handlers must be careful to avoid becoming entangled if a dolphin rolls in the net. When handling dolphins, care should be taken to avoid sensitive areas such as the rostrum, eyes, blowhole and mouth.

Dolphin in Distress – Several in-water veterinarians are on site and available to render assistance if a dolphin appears to be in distress. Call for a veterinarian if a dolphin exhibits signs of injury, extreme stress or shock. If necessary, they will immediately assess and provide treatment for shock. Each veterinarian carries and is responsible for one of four Dolphin Emergency Treatment Kits (in yellow waterproof Pelican Cases) (Fig. 2). These kits are assigned to veterinarians aboard the Catch and Chase Boats.

Figure 2. Dolphin Emergency Treatment Kits

Cutting the Net – This is a last resort used to free a person or dolphin and will only be done in an emergency or when directed by the CM or RCA. Specially designed net knives (Fig. 3) are available and are employed as needed. If a dolphin or person is severely entangled in the net, one should attempt to gather slack in the net and alert the CM, RCA and/or MSO.

Figure 3. Net knife

Pulling the Net – After each net set, decisions will be made as dolphins are secured, held stationary and moved outside the compass to the Dolphin Processing Boat. Listen carefully for instructions when you are called to help maneuver and/or secure points on the net compass. Pulling, cleaning and stacking the net can be done once all dolphins are safely restrained and secured outside the compass. When it comes time to pull, clean and stack the net back aboard the Catch Boat you will be instructed to do so by the Catcher.

DOs and DON'Ts

Remember, common sense prevails. Use good judgment and remain alert and be aware of your surroundings at all times. If you are hurt or need assistance make sure to call out to alert those around you.

Personal Effects – Bracelets, watches, necklaces and rings can cause injury to both people and dolphins. Such items are easily caught in the net and can also be lost. Remove and secure ALL jewelry and personal belongings (*i.e.,* cell phones, cameras and PDA's) BEFORE boarding each morning.

"NO" or "JUMP" – The call for anyone to go overboard for any reason and from any vessel is given **ONLY** by the boat captain. To avoid ANY possible confusion during times of rapid deployment during C-R operations, boat captains will call "NO" to hold fast and "JUMP" to indicate it is safe to go overboard. This helps eliminate the potential for confusing the commands "NO" and "GO".

Diving – Under NO circumstances shall anyone dive into the water. Use a boat ladder or gently ease yourself overboard to avoid the risk of serious head, neck and/or spinal injury and to minimize stress on captured dolphins.

Noise – Please refrain from excessive talking and noise while C-R operations and sampling of dolphins is underway. Do not distract boat captains and keep a watchful eye, especially while boats are in-motion and while handling restrained dolphins. Boat captains, in-water personnel and especially those in close proximity to the Dolphin Processing Boat, should speak quietly and softly while dolphins are being processed. Boat captains also need to consider the underwater noise/pitch of propellers and the adverse effects that may have on dolphins that are being restrained nearby.

Smoking – Smoking is a fire hazard, offensive to others and risks contamination of samples in an open processing environment. A no-smoking rule will be in effect aboard all vessels, around fuel docks and in any/all areas that are in close proximity to others.

Footwear – Anyone entering the water does so at their own risk. Hard-sole footwear (*e.g*., diving booties) is required. The varied marine bottom has certain hazards ranging from oyster/clam shells to broken glass and venomous rays and skates. In this environment, it is advisable to walk slowly and shuffle one's feet in order to avoid stepping on underwater hazards. If stung or injured, notify a supervisor or medical personnel immediately. A stingray treatment kit, consisting of a thermos of hot water, medical provisions and a 'soaking boot' is brought along during field operations. Also, in some areas jellyfish and sea-lice pose risks. People who are allergic to bee stings are at a higher risk and need to advise the CM, RCA and MSO if any such allergic reactions are possible.

Cell Phones and Cameras – No unauthorized use of cell phones or cameras will be allowed. Cell phones should be turned off during on-water operations and any/all images taken are subject to review and approval of Dr. Bossart and NMFS.

Personal Comfort – **Rehydrate often.** Prolonged exposure to the elements and exhaustive work efforts involved in the process of C-R requires special considerations and caution. **Use ample sunscreen** (waterproof SPF 15 or higher) and protective clothing (hats, long sleeved shirts, polarized sunglasses, etc.) to protect against sunburn and discomfort. Water coolers are placed on-board each boat and everyone is encouraged to drink plenty of fluids. Long days in the hot sun are exhausting. STAY ALERT and get plenty of sleep during off hours.

Personal Responsibility – At all times, participants are expected to exhibit responsible behavior and a professional attitude. Participants are not only representatives of their respective institutions or agencies they are also representatives of the hosting organization(s). Due to the importance and inherent risks associated with C-R operations, participants are expected to get essential rest and nourishment.

Dolphins in Distress

In the event of a dolphin-related emergency, follow the instructions of the veterinarian in charge and support directives of supervisors and senior dolphin care staff. Provide ample room for staff to respond and stand by to assist if called upon.

Dolphin Emergency/Emergency Release – If deemed necessary by the primary veterinarian, assisting veterinarian or senior dolphin care staff, a dolphin that appears to be in distress must be immediately returned to the water from the Dolphin Processing Boat. If, upon return to the water, the dolphin's condition does not improve, the primary veterinarian will decide whether to release the dolphin or continue to hold it for further treatment. If the dolphin is released, a support (photo-ID) boat may be used to track and monitor the dolphin until normal behavior is observed.

Dolphin Treatment or Euthanasia – In the event that the primary veterinarian determines that a dolphin would benefit from short-term care to aid in recovery from an injury or disease, then that dolphin will be transferred to shore and taken to the nearest approved marine mammal treatment center. The RCA will coordinate transport logistics as directed by the NMFS Regional Stranding Coordinator. If, due to injury or pre-existing condition, the primary veterinarian deems it necessary to euthanize a dolphin and, following consultation with and approval from NMFS staff, then the dolphin will be humanely euthanized through acceptable medical protocols.

Figure 4. Emergency transport vehicle

Dolphin Death – In the unlikely event a dolphin expires during C-R operations, the Permit Holder will be immediately notified and the Senior Veterinarian will secure the carcass for an immediate and well-documented necropsy. The Permit Holder or assigned representative will immediately notify the appropriate NMFS personnel.

Disease Transmission

Disinfectants and Special Handling of Dolphins with Suspected Zoonoses – Some captured dolphins may potentially harbor a zoonotic disease (or diseases). In such cases, contact will be limited as directed by the PI or senior veterinarian. Those with open wounds, cuts or sores should refrain from direct contact with these dolphins. All other dolphin handling staff should take added precautions and utilize protective clothing (*i.e.,* gloves, surgical masks, rash guards, eyewear, etc.). Following a dolphin's treatment and evaluation, all persons (and equipment) that may have had contact with the dolphin will be isolated to an area away from other personnel and dolphins (downstream and downwind) and begin disinfecting with approved materials, being careful to avoid contact with the eyes and/or other sensitive areas of the body. Clothing (rash guards, shirts, etc.,) should be removed and soaked for a minimum of 5 min in a disinfectant solution or, as directed by a veterinarian. To avoid possible contamination from dolphin to dolphin, the Dolphin Processing Boat is thoroughly cleaned and disinfected immediately following each individual dolphins examination.

Quarantine Protocol/Marine Animal Park Personnel – As the Dolphin HERA Project involves the capture and sampling of wild dolphins, there is a possibility of disease transmission between public display dolphins and the wild bottlenose dolphin stock that we will be sampling, and vice versa. This is especially relevant for all marine animal industry personnel (trainers, vets, animal care, etc.) who are coming to, or from, a park or zoo to assist in C-R activities. Therefore, all Dolphin HERA Project participants are advised to remain (dolphin) contact free prior to participating, or after participating, in C-R fieldwork, for a minimum period of 72 hours. All clothing and equipment brought to or taken from the C-R should be washed and disinfected.

Media/Interference/Public Concern

Operating under permit from NMFS, we are conducting scientific studies to benefit free-ranging populations of dolphins near CHS and in the IRL. The protocols we establish here will be used elsewhere for the same purposes. Please refer all media inquiries to the PI or CM and refrain from making any statements on your own. If in the event intervention or explanations are required to any concerned third parties, the PI and CM will direct those efforts.

ACKNOWLEDGEMENT AND ACCEPTANCE OF PARTICIPANT in DOLPHIN HERA PROJECT

BY MY SIGNATURE BELOW EXECUTED THIS _____ DAY OF _____, 2006, I ACKNOWLEDGE THAT I HAVE CAREFULLY READ AND DO UNDERSTAND ALL OF THE ABOVE-STATED SAFETY PROVISIONS OF THIS DOCUMENT AND THAT I KNOWINGLY AND VOLUNTARILY AGREE TO THESE HAZARDS, CONDITIONS AND RESPONSIBILITIES AS OF THE DATE INDICATED ABOVE.

PARTICIPANT:

_____	_____
Witness	Signature:
_____	_____
Witness	Printed:

HERA BOAT CAPTAIN'S DAILY CHECKLIST

1. Confirm vessel is clean and fully equipped with required USCG safety equipment.
2. Check vessel fuel, oil, all electronics (VHF, GPS, depth sounder, lights) and any maps and charts).
3. Ensure all C-R equipment (depth poles, stern anchors, etc.) is stowed and secured.
4. Confirm your vessel VHF call sign and the working channel and review your assignment with the CM/RCA.
5. Confirm crew assignment and make sure each participant has an assigned PFD and all necessary gear.
6. Review chain of command. (boat captain, RCA, CM, PI).
7. Identify any crewmembers with CPR and/or first aid training.
8. Determine if any crewmembers have allergies or special needs.
9. Make sure all personal items, food and water coolers are on-board and stowed.
10. Review Emergency Response Plan (ERP) and SOP.
11. Review following list of DOs and DON'Ts:

- DO respect the net
- DO stay in sight of boat captains
- DO wear booties
- DO stay alert and listen to supervisors
- DO listen to "no" or "jump" commands
- DO use boat ladders to reenter a boat NOT propeller shafts or lower units
- DO use caution and be quiet around dolphins
- DO rehydrate often
- DO use sunscreen
- DO wear protective clothing
- DO use caution on docks and boat ramps
- DO refuel and clean boats nightly
- DO use on-board restroom facilities
- DO be respectful and courteous
- DO NOT dive into water
- DO NOT wear any jewelry, rings or watches
- DO NOT go near propellers
- DO NOT get between rafted boats
- DO NOT make excessive noise
- DO NOT smoke
- DO NOT bring cell phones or cameras
- DO NOT talk to the media
- DO NOT be afraid to ask questions

APPENDIX B

Dolphin HERA Project – Dolphin Sampling Guide

***** NOTE: morphometrics, photography and ultrasound will take place during animal processing*****

Blood (** FILL ALL TUBES COMPLETELY):

- Trace element Sarstedt Lavender tube #1, then #2 needs to be drawn FIRST!
- Any order for blood tubes: Lavender, Yellow, Green, Tiger, RNA
- Last tube is I-STAT (remove needle and draw 0.2 mL of blood into lithium heparized syringe directly from butterfly catheter after all other blood tubes have been drawn; discard needle; insert supplied black top on syringe until sample analysis can be performed)

* **RNA – IMPORTANT: Do not contaminate tubes – ALWAYS handle tubes with gloves and/or plastic bags (hands contain RNAase or enzymes which degrade RNA).**
* **Gently invert tubes 10 times; invert I-STAT syringe**

Urine:

- Sterile gloves (size 7.5 or 8.5) are presented to veterinarian
- Dry gauze is used to dry genital area
- KY jelly is applied to catheter (8 Fr x 22 in for male, 10 Fr Self-catheter for female)
- Urine sample is collected in conical vial (50 mL)

* **Have conical vial in hand during procedure as dolphin may urinate prior to inserting catheter.**

Fecal:

- 6 swabs are taken (3 culturettes, 3 dry swabs), directly in rectum
- Draw up 15 cc of saline in 20 cc syringe using 18 gauge x 1½ in needle
- Remove needle and attach to 14 Fr Self-catheter
- Apply KY jelly to Self-catheter
- Contents of syringe (after taking fecal sample) are emptied into conical vial
- Solid sample taken opportunistically

* **Have fecal sample container available during processing, often later in processing the dolphin will defecate voluntarily.**

Blowhole:

- Dry gauze pad is used to dry blowhole
- As the dolphin takes a breath, 4 swabs (2 culturettes, 2 dry swabs) are simultaneously inserted into blowhole
- Using plastic sterile Petri dish collect blow fluid and snap cover afterwards
- Also collect sample from blowhole discharge if available during processing (mainly looking for *Nasitrema* ova)

Gastric:

- Two mouth towels are prepared for dolphin handlers to open the dolphin's mouth
- KY jelly is applied to stomach tubes
- Stomach tube is inserted into mouth and gastric sample is collected into two conical vials (15 mL)

Blubber biopsy:

- Two 10 cc syringes are prepared, each with 10 cc of bottled lidocaine with epinephrine
- 19 gauge x 1½ in needles are used to draw lidocaine from bottles, and replaced with new 19 gauge x 1½ in needles for injection
- Biopsy site is prepped for injection using chlorhexiderm scrub followed by methanol rinse
- Five min after anesthesia injection, biopsy site is prepped again for blubber extraction (chlorhexiderm scrub and methanol rinse)
- The scalpel handle and forceps are removed from aluminum foil (avoid skin-to-instrument contact) and a #10 sterile scalpel blade is attached to handle
- Blubber sample is placed in unwrapped Petri dish
- Gauze soaked in ferric subsulfate is used to promote clotting at the biopsy site

Tooth:

- Fill two injector guns with one ampule of carbocaine each *(these should be pre-loaded)*
- Attach a dental needle (blue cap, clear base) to the injector gun
- Be certain that a dry towel is available to dry target tooth area on lip
- Unwrap gelfoam and place in tooth tackle box (2-3 pieces are used for each tooth)
- Sharpie marker is used to mark tooth to be removed (#15)
- Carbocaine is injected into periodontal ligament for local anesthesia of tooth
- Dental elevators are used to cut the ligaments surrounding the tooth
- Once ligaments are cut, an extractor is used to loosen and remove the tooth
- Prior to tooth removal, gelfoam pads should be rolled tightly
- Gelfoam rolls are placed in empty tooth socket to promote clotting (2-3 rolls per socket)
- Tooth is placed in plastic vial
- Dental tools are cleaned with chlorhexiderm scrub and isopropyl alcohol between each dolphin
- Dental elevators must be sharpened each evening

Rototag/Dorsal fin plug:

- One injector gun is filled with one ampule of lidocaine-epinephrine
- Attach a short needle (yellow cap, clear base) to the injector gun
- The rototag site is prepped for injection using chlorhexiderm scrub and methanol rinse
- Lidocaine-epinephrine is injected into dorsal fin (triangular pattern) to provide anesthesia

- Clean the #2 sharpened corkborer, rototag and applicator with isopropyl alcohol
- Load rototag into applicator (blue rototags for males, pink rototags for females). The piercing piece of the tag should be facing the larger hole of the applicator.
- The #2 corkborer is used to bore a hole in the trailing edge of the dorsal fin.
- The dorsal fin plug is removed by pushing the #1 through the #2 corkborer which, in turn, pushes the sample into the designated vial.
- The rototags are placed into the hole in the dorsal fin and the number of the tag is recorded by the Data Recorder.

APPENDIX C

Wild Dolphin Health Assessments
Two-Veterinarian Guidelines

Prepare Dolphin Processing Boat (wash and disinfect mats and prepare scale), weigh dolphin, move dolphin onto Dolphin Processing Boat, put Bimini top up and conduct dorsal measurements

Veterinarian 1 (HEAD): Immediately after the dolphin is moved onto the Dolphin Processing Boat, careful observation for frequency and quality of respirations, eye movement, vocalizations and general attitude of dolphin is made for signs of undue stress in the initial acclimation to the processing.

- Primary physical exam (on-going throughout dolphin processing)
- Blowhole sampling
- Gastric sampling
- Tooth anesthesia
- Tooth count
- Tooth extraction
- Blubber ultrasound

Veterinarian 2 (REAR): Awaits approval from Vet 1 to roll dolphin onto right side to begin sampling.

- Urine
- Milk
- Fecal
- Blubber anesthesia (5 min)
- Dorsal fin photos*****
- Dorsal fin anesthesia for rototag*****
- Attach rototag*****
- Photos of dorsal fin and rototag(s)*****
- System ultrasound **L** (waiting for blubber anesthesia, 5 min)
- Blubber biopsy
- Freeze brand right dorsal fin (in water unless last dolphin to record)*****
- System ultrasound **R** during freeze branding on **L** side
- Post-bleed on Dolphin Processing Boat if another dolphin is not waiting to be processed
- Replace dolphin back in water, remove stretcher - post-bleed if not already completed
- Girth measurements prior to release (can also be measured while waiting to come aboard)
- Release by having 3 people on one side and on count of 3 have them push the dolphin away and step back away

**can be done on Dolphin Processing Boat or while dolphin is being held in water*

<u>Short Processing (due to young age, advanced pregnancy or inclement weather) - in water):</u>

- Primary physical exam (on-going throughout dolphin processing)

- Blood sampling
- Dorsal fin anesthesia and rototag attachment
- Freeze branding
- Measure length/girths
- Dorsal fin photos
- Additional sampling - possible, depending on circumstances
    - Blowhole, Fecal, Milk
    - Blubber biopsy; lesion biopsy

APPENDIX D

National Institute of Standards and Technology Bottlenose Dolphin Capture-Release Protocol for Collection of Blubber, Skin, Blood and Milk Samples for Contaminant Analysis

Biopsy Protocol for Blubber and Epidermis

Preface:

Inadvertent contamination during sample collection and handling is the major concern during the biopsy procedure. There are two major sources of contamination:

1. Hydrocarbons from sources other than the dolphin. These include:
   - Sunscreen
   - Boat fuel
   - Insect repellent
   - Other oils associated with the Dolphin Processing Boat
   - Cigarette smoke
2. Carry-over contamination from the previous dolphin. The concentrations of organohalogen pollutants in dolphins can vary by nearly two orders of magnitude between multiparous females (very low) and juveniles or old males (very high). Ensure that instruments are not reused between dolphins.

Materials:

- Stainless steel scalpel handle, pre-cleaned with acetone and hexane, wrapped in hexane-rinsed aluminum foil and autoclaved (2/dolphin)
- Sterile stainless steel scalpel blades kept in manufacturer's foil wrapper until biopsy is performed or sub-sampled (2/dolphin)
- Stainless steel forceps, pre-cleaned with acetone and hexane, wrapped in hexane-rinsed aluminum foil and autoclaved (2/dolphin)
- Glass Petri dish with cover, pre-cleaned with acetone and hexane, wrapped in hexane-rinsed aluminum foil and autoclaved (1/dolphin)
- HPLC or pesticide residue grade methanol (in clean Teflon squirt bottle, NIST will provide)
- Sterile individually wrapped 4x4 gauze (2/dolphin)
- Teflon jars (15 mL), pre-cleaned by NIST
- Lid labels
- Corning external thread 2 mL plastic cryovials (orange cap)
- Nalgene jar
- Cryopen (Sharpie marker)
- Deionized water (in clean Teflon squirt bottle, NIST will provide)
- Deionized water (in regular plastic bottle, NIST will provide)

Procedure/Special Considerations:

Blubber Sampling for Perfluorinated Compounds: The major consideration regarding contamination is that products made of Teflon may contain some of the perfluorinated chemicals of interest in this study. Therefore, Nalgene plastic jars will be used for storage of the samples. Cut a sub-sample of the blubber equivalent to 0.1-0.2 g (or a cube of approximately 0.5 cm on each side) from the **full thickness** of the blubber layer then **place this sub-sample in a 15 mL Nalgene jar**. Check to make sure the container is properly labeled with a cryopen. Place the sample in the liquid nitrogen vapor shipper.

Blubber Sampling for Organochlorine and Organobromine Compounds: Using forceps, place the remaining blubber biopsy in the 15 mL Teflon jar. Check to make sure the jar is properly labeled with a lid label and that the label is secure. **Do not** use common lab tape to label the jar; this will either disintegrate or fall off in the liquid nitrogen vapor shipper. Be aware that many types of plastic will not withstand the temperatures present in the liquid nitrogen vapor shippers. Place the jar with the biopsy sample in the liquid nitrogen vapor shipper.

Epidermis for Trace Elements: Avoid contact with metal and all 'unclean' surfaces, (*i.e.*, everything other than the pre-cleaned biopsy instruments and the interior of the containers into which the samples will be deposited). Place section of epidermis in a pre-labeled 2 mL cryovial and other sections of epidermis as requested.

Prior to biopsy collection, kits containing a scalpel blade and handle, forceps and a glass Petri-dish were prepared by NOS staff in the NIST laboratory. All items were rinsed with acetone, then hexane, covered with hexane-rinsed aluminum foil and autoclaved.

Biopsy procedure

Blubber thickness was measured at the biopsy site using ultrasound. The biopsy site was wiped with chlorhexiderm-soaked gauze, rinsed with methanol and injected with lidocaine with epinephrine. A blade was placed onto the pre-cleaned scalpel handle. Just prior to cutting the biopsy, the area was again wiped with chlorhexiderm-soaked gauze and then rinsed with methanol. Using the pre-cleaned instruments, an approximately 3-4 cm diameter full thickness (*i.e.*, down to but not including the muscle layer) biopsy was taken and placed in the Petri dish, covered and returned to the Sample Processing Boat. With the biopsy in the Petri dish, the epidermis was dissected from the blubber using a new solvent-rinsed scalpel handle, blade and forceps. The following information should be recorded: name of person taking the biopsy and person subsectioning the biopsy sample, dimensions of the biopsy and whether the biopsy is full thickness. The blubber is then sectioned through its **full thickness** and stored in the following order:

1. Perfluoro compounds (Nalgene 15 mL jar)
2. Organochlorine and organobromine compounds (Teflon jar 15 mL)
3. Others (*i.e.*, fatty acids, histology, biomarkers, etc.)

All surfaces of the epidermis were rinsed with deionized water (provided by NIST) from a Teflon wash bottle. The epidermis was sub-sectioned into pieces and stored in the following order:

1. Trace elements (2 mL cryovial)
2. Others (*i.e.*, biomarkers, etc.)

Biopsy Sub-sample Summary (in order of collection):

Order	Tissue	Container	Analyte(s)	Researcher(s)	Storage
1	Blubber	15 mL Nalgene Jar	Perfluoros		Liquid Nitrogen
2	Blubber	Teflon Jar	Organochlorine/bromine		Liquid Nitrogen
3	Blubber	Others	Others	Others	Varied
4	Epidermis	Cryovial	Trace Elements		Liquid Nitrogen
5	Epidermis	Others	Others	Others	Varied

***Avoidance of sample contamination is crucial. This includes contamination from one vial to another. Hence, it is important to adhere to the proposed sample order and, if possible, avoid touching the forceps to sample containers or any other item that might contaminate the sample.**

After tissue has been sub-sampled, sample blanks should also be taken on-board the Sample Processing Boat. Take blanks of each sample below for each researcher until at least 3-4 blanks of each sample are collected. Blanks should be made in the same manner as samples were collected or handled. (*e.g.*, if the veterinarian does not wear gloves during the lidocaine injection, gloves do not need to be worn to make the lidocaine blanks).

Boat Blanks:

Analyte(s)	Container(s)	Researcher(s) (# blanks/day)	Storage
Lidocaine with Epinephrine	2 mL Cryovial 2 mL Cryovial Teflon Jar Others	Perfluoros _____ Trace Elements _____ Organochlorine/bromine_____ Others _____	Liquid Nitrogen* Varied
Methanol from Teflon Squirt Bottle	2 mL Cryovial 2 mL Cryovial Teflon Jar Others	Perfluoros _____ Trace Elements _____ Organochlorine/bromine_____ Others _____	Liquid Nitrogen* Varied
Millipore Water from Teflon Bottle	2 mL Cryovial Others	Trace Elements _____ Others _____	Liquid Nitrogen* Varied

***Storage of blanks in liquid nitrogen is not necessary but doing so maintains consistency between samples and blanks and will aid in keeping appropriate blanks and samples together.**

Lidocaine with Epinephrine Blanks:

Draw lidocaine out of medical bottle using a clean, sterile plastic syringe and needle and squirt into vials.

Date made: (A) (B) (C)

Millipore Water Blanks (*spread out over capture):

Rinse water from clean Teflon bottle used to rinse epidermis.

Date made: (A) (B) (C)

Methanol Blanks (*spread out over capture):

Squirt methanol from clean Teflon squirt bottle used to rinse epidermis prior to biopsy.

Date made: (A) (B) (C)

Storage of Blubber, Skin and Blanks:

Please note how samples were stored in the field, back at the lab and how they were shipped.

Blood Sampling

Preface:

Several studies have shown that plasma is an appropriate blood fraction to use for the analysis of organohalogen pollutants and respective metabolites. Plasma is obtained from centrifuged blood collected in tubes containing an appropriate anti-coagulant; in this case sodium heparin will be used. Heparin may contain contaminants; hence, blank tubes will need to be prepared during sample collection. Four 10 mL tubes of blood (ca. 6 mL plasma) are adequate for the determination of organochlorine/organobromine and perfluorinated compounds.

Figure 1. Blood kit

Two 7.5 mL plastic lavender-top tubes will be used to collect blood for trace element analysis. These tubes **MUST BE USED FIRST** before all other blood is collected from the dolphin as the tops of other tube types can be a source of trace element contamination. These tubes should also be collected in the order that is written on the tubes (1-2). As above, separate blood collection devices should be used for each dolphin.

Materials:

- 10 mL green-top sodium heparin blood tubes (4/dolphin)
- 7.5 mL lavender-top EDTA KE blood tubes (2/dolphin)
- Centrifuge
- Refrigerator or cooler with ice and bubble wrap
- Blood sampling device (butterfly)
- Deionized water (in Teflon bottle, NIST will provide)

- Deionized water (in regular plastic bottle, NIST will provide)
- NIST pre-cleaned Teflon jars (1/dolphin)
- Corning external thread 5 mL plastic cryovials (orange cap) (2/dolphin)
- Lid labels
- Teflon 10 mL round bottom tubes (1/dolphin)
- Labels for Teflon tube made from business cards
- Paper towels
- 50 mL plastic centrifuge tubes with outside labels (5/dolphin)
- Acetone/hexane rinsed Pasteur pipettes (1/dolphin)
- Pipette bulbs
- Acetone/hexane rinsed 10 mL glass serological pipettes (1/dolphin)
- Pipette-Aid (electronic or roll-ball)
- Plastic pipettes (1/dolphin)
- Freezer (ca. -20^{o}C and -80^{o}C) or cooler with dry ice
- Test tube racks

Procedure:

The blood collection site on the flukes is wiped with a surgical scrub (2% chlorhexidine gluconate) and then with a methanol-soaked gauze pad.

Trace Elements: Collect two 7.5 mL tubes of blood into the plastic lavender-top tubes labeled #1 and #2 before all other blood and collect in the appropriate order. Invert 8-10 times to mix the EDTA with the blood. On the Sample Processing Boat, keep these tubes cool until processed. Record the lot number and volume of these tubes. Place each blood tube inside a 50 mL centrifuge tube with an outside label and freeze upright on dry ice. **DO NOT PLACE THE TUBES IN THE LIQUID NITROGEN VAPOR SHIPPER UNTIL SHIPPED.**

Organochlorine and Organobromine Compounds: Collect four 10 mL tubes of blood into the green-top tubes. After collection, invert the tubes 8-10 times to mix the blood and the heparin to ensure that the sample does not become clotted. Keep these tubes cool until processed. Do not place in direct contact with the ice as hemolysis can occur.

In the field, use the centrifuge to spin the tubes at 1200 rpm for 15 min. For **perfluorinated contaminants and PCB metabolites** samples, transfer plasma of two 10 mL green-top tubes into two plastic 5 mL cryovials using disposable **plastic pipettes** (to avoid possible contamination by Teflon). Place cryovials in liquid nitrogen vapor shipper. Retain the remaining cellular material in the original tubes. Wrap tubes with paper towel and label the outside with freeze brand, date, remaining cells, researcher and location (IRL or CHS). Freeze upright on dry ice.

Combine the plasma samples for organochlorine and organobromine compound analysis from the two remaining green-top tubes into a Teflon jar using hexane-rinsed glass Pasteur pipettes. Measure and record the total volume of plasma using a hexane-rinsed 10 mL glass serological pipette. Mix the plasma using the Pipette-Aid 4 times and transfer 2 mL to a pre-cleaned, tared 10 mL Teflon tube. Record the Teflon tube number for each dolphin. Wrap this tube in a paper towel and place it back into its 50 mL centrifuge tube

containing a label with the tube tare weight and the sample information. Secure the lid, check the label and then place the Teflon jar into the liquid nitrogen vapor shipper and the tube into dry ice. Record the blood tube lot number and the volume of the remaining cells in the green top tube. Retain the remaining cellular material in the original tubes by wrapping these tubes in a paper towel, placing them in a 50 mL tube with an outside label and freezing them upright on dry ice.

Blood Blanks

Collect three field blanks for perfluorinated compounds in plasma, three field blanks for organochlorine and organobromine compounds in plasma and three field blanks for trace elements.

Blanks should be made in the same manner as samples were collected or handled. (If the veterinarian does not wear gloves during blood collection, gloves do not need to be worn to make the blood blanks.)

Green Lot _____ A, B, C date _____
Lavender Lot _____ A, B, C date _____

Organochlorine and Organobromine Compounds in Plasma and Trace Elements Blanks:

1. Using a fresh blood sampling device, draw deionized water from the Teflon bottle into 2 lavender-top tubes (A1 first and then B1) and then 2 green-top tubes. Invert the tubes as done with the blood sample. Centrifuge the green-top tubes similar to what was done for the blood.
2. Label the lavender-top tube 'Blank A1, date, whole blood, researcher/location'. Place inside a 50 mL tube and freeze upright on dry ice.
3. Transfer two-thirds of the water from the top of the green-top tubes (volume similar to plasma) into a Teflon jar via a glass Pasteur pipette (as you would transfer the plasma). Mix this water 4 times and transfer 2 mL into a tared Teflon tube. Wrap the Teflon tube in a paper towel and put it back inside its 50 mL centrifuge tube along with the label containing the tube tare weight and 'Blank A, Date, Plasma sub-sample, researcher/location'. Label the Teflon jar 'Blank A, Date, Plasma, researcher/location'. Place the Teflon jar in the liquid nitrogen vapor shipper and the tube upright on dry ice. Label the green-top tubes with 'Blank A, Date, remaining cells, researcher/location', wrap these in paper towels, place them inside 50 mL centrifuge tubes and freeze upright on dry ice.
4. Repeat steps 1 through 3 twice more, using new tubes and blood sampling devices. Label these appropriately blank B and C for whole blood and plasma.

Perfluorinated Compounds in Plasma Blanks:

Green Lot _____ A, B, C date _____

1. Label 3 green-top tubes 'Remaining cell blank X, date, researcher/location' with A, B or C being substituted for 'X'. Label three 5 mL cryovials 'Plasma Blank X, date, researcher/location'.
2. Using a fresh blood sampling device, draw deionized water from the regular plastic bottle into 3 green-top tubes in the order of A, B, and then C. Invert and centrifuge as was done with the blood sample. Transfer the top two-thirds of water (volume similar to plasma) using a plastic pipette into the appropriately labeled 5 mL cryovials. Freeze the cryovials in liquid nitrogen.
3. Pour water from a polyethylene plastic bottle into three 5 mL cryovials and label 'Water for plasma blank X, date, researcher/location' with A, B or C being substituted for 'X'. Place the cryovials into the liquid nitrogen vapor shipper.
4. Dispose of green-top tubes with remaining water.

NOTES: (1) Only one set of blanks (3 Teflon jars, 3 Teflon tubes, three 10 mL cryovials, and 3-lavender-top tubes) will be collected for each capture event, unless samples are taken from different lots of blood tubes. Therefore, collect the blanks on one day of the capture event and label appropriately (see Labeling Section below). (2) Check to see if blood tubes are all from the same lot. If tubes are from different lots, run three blanks on each lot. (3) Be sure to keep the green-top tube blanks with the green-top blood samples; likewise for the lavender-top tubes.

Blood Collection Summary:

Order	Tube Type	Anticoagulant	Tube Volume	# Tubes	Blood Fraction	Short-Term	Long-Term	Investigator/Analyses
1	Plastic Lavender	EDTA KE	7.5 mL	2/dolphin	Whole Blood	Keep in Tube	Liquid nitrogen	_____ Trace Elements
2	Green Top	Heparin	10 mL	2/dolphin	Plasma	2 - 5 mL orange capped Cryovials	-80°C ship in liquid nitrogen shipper	_____ Perfluoros and Organohalogen metabolites
3	Green Top	Heparin	10 mL	2/dolphin	Plasma	1 - 15 mL Teflon jar 1 - 10 mL Teflon tube	Liquid nitrogen -80°C ship on dry ice	_____ Organochlorine and Organobromine Compounds
Others								
Blanks	Plastic Lavender	EDTA KE	7.5 mL	6/session	Blank	Keep in Tube	Liquid nitrogen	_____ Trace Elements
Blanks	Green Top	Heparin	10 mL	3/session	Blank	3 - 5 mL Cryovials	-80°C ship in liquid nitrogen shipper	_____ Perfluoros and Organohalogen metabolites
Blanks	Green Top	Heparin	10 mL	6/session	Blank-Plasma	3 - 15 mL Teflon Jars 3 - 10 mL Teflon tubes	Liquid nitrogen -80°C ship on dry ice	_____ Organochlorine and Organobromine Compounds

Storage of Blood and Blanks:

Please note how samples were stored in the field and at the lab and how they were shipped.

Milk Samples

Preface:

The major sources of contamination of the milk sample is from the sample collection device and carry-over of previous milk samples if the device is re-used between dolphins. The milk collection device should not be shared among dolphins.

Materials:

- Milk sampling device (1/dolphin)
- NIST pre-cleaned Teflon jars
- Lid labels
- Orange cap Corning external thread 5 mL plastic cryovials
- Deionized water (in Teflon bottle, NIST will provide)
- Deionized water (in polyethylene plastic bottle, NIST will provide)

Procedure:

Collect the milk sample and return to Sample Processing Boat in sampling device. Dispense milk into the 15 mL Teflon Jar (organochlorine/organobromine), 5 mL cryovial (Trace Elements), 5 mL cryovial (PFCs) and other sample containers. Aliquots should not be removed from the contaminant study containers because of the risk of sample contamination. Do not overfill the containers; make sure there is room left to allow for the expansion of the sample due to freezing. Ensure that the sample is properly labeled with a lid label and that the label is secure. Again do not use regular lab labels or tape on the Teflon jar. Place the jars and cryovials in the liquid nitrogen vapor shipper.

Blanks for Organochlorine and Organobromine Compounds and Trace Elements in Milk:

Using a clean, unused sampling apparatus, pour a volume of deionized water from the Teflon bottle into the device that is comparable to a milk sample. Dispense the deionized water into a Teflon jar and 5 mL cryovial labeled 'Milk field blank, date, researcher/location'. Collect three field blanks in this manner using a new sampling apparatus for each, labeled A, B, and C.

Made blanks A, B, and C on _____.

Organohalogen Metabolites and Perfluoro Compounds in Milk Blanks:

Using a clean, unused sampling apparatus, draw an amount of deionized water from the regular plastic bottle that is comparable to a milk sample. Dispense, in order, the deionized water from this single apparatus into three 5 mL orange cap cryovials, labeled 'Milk blank-A, B, and C, date, researcher/location'.

Made blanks A, B, and C on _____.

Storage of Milk and Blanks:

Please note how samples were stored in the field and at the lab and how they were shipped.

Labeling

The Teflon jars, cryovials and blood tubes should all be labeled with a standard format. Each lid label or tube should include the following information:

- Freeze Brand #
- Date sampled (ddMmmyy)
- Tissue Type (if multiple samples/tissue collected, 1, 2 or 3 should follow)
- Institution/Location of Dolphin (IRL or CHS)

Example: FB 999
05Feb03
Plasma
NIST/IRL

For field blanks, each jar or tube should have the following information:

- Blank-ddmmyy (date of the collection, *i.e.*, if the collection date is 5 February 2003 the number to follow would be 020503)
- Date sampled (ddMmmyy)
- Blank Type (if multiple blanks collected, A, B, or C should follow)
- Institution/Location of Dolphin (IRL or CHS)

Example: Blank-020503
05Feb03
Plasma-A
NIST/IRL

Shipping

The cryovials, Teflon jars, Nalgene jars and lavender-top tubes containing the blubber biopsy, milk samples, whole blood and plasma samples will be shipped in a liquid nitrogen vapor shipper. The remaining blood cells and plasma sub-samples will be shipped on dry ice. All samples must be shipped Federal Express, Priority Overnight to the following address:

Rebecca Pugh
NIST - Hollings Marine Laboratory
331 Fort Johnson Rd.
Charleston, SC 29412
(843) 762-8952

Please provide a copy of the data sheets along with the shipment. Please contact Rebecca Pugh prior to shipping to ensure that she will be there to accept the shipment. If you have questions, contact Rebecca at (843)762-8952.

APPENDIX E

Bottlenose Dolphin Freeze Brand Application Procedure

1.0 OBJECTIVE

Detail procedure for freeze branding the dorsal fins of bottlenose dolphins.

2.0 PERSONNEL/TRAINING/RESPONSIBLITIES:

Only personnel trained in these techniques may perform these procedures. Caution must be used when working with liquid nitrogen - it is a dangerous material that can splatter while being poured, possibly causing freeze burns. Therefore, appropriate protective equipment, such as goggles and cryogenic gloves, should be used whenever working with liquid nitrogen. ***All safety procedures outlined below must be followed at all times*.**

3.0 MATERIALS

(22) 2½ in branding irons (numbers 0-9, letters A, F, J, K, M, N, R, T, V, W, X and Y, all with handles, Stone Manufacturing, Kansas City, MO)
(2) 9/16th combination wrenches
(2 sheets) 60 grit sandpaper
(2 sets) Cryogloves (size appropriate; Fisher Scientific, Pittsburgh, PA)
(1) Taylor-Wharton LD25 or LD10 cryogenic Dewar (filled with liquid nitrogen; www.taylorwharton.com)
(1) Insulated 5 gal plastic bucket
(1) Freeze brand order matrix
(1) Stopwatch
(~10) Hand towel(s)
(4) Sponges (optional)
(2) 2 gal plastic buckets (optional)
(1) Canon EOS1D 35mm digital camera (similar cameras can be substituted)

4.0 GUIDELINES

4.1 Number Selection

4.1.1 Dolphin(s) should be sexed by a veterinarian or experienced handler as soon as it is safe and practicable.

4.1.2 Relay dolphin(s) sex to personnel handling the dolphin and to the Data Manager on the Sample Processing Boat.

4.1.3 Branding irons are selected after reviewing the freeze brand order matrix to determine the next available number (see Section 5.1 for the sequence of available freeze brand numbers) and based on the sex of the dolphin to be branded (females receive odd numbers, males receive even numbers).

4.2 Cooling of brands

4.2.1 Inspect the face of the irons for rust or grit; if necessary sand the iron face(s) until smooth.

4.2.2 Place the irons in the insulated bucket, handle-end up.

4.2.3 Put on cryogloves.

4.2.4 Decant liquid nitrogen from the Dewar into the bucket, taking care to avoid spills or splashing of the liquid. This step should be done away from co-workers in case the liquid nitrogen splashes.

4.2.5 Enough liquid nitrogen should be decanted to fill the bucket to a depth of 3-4 in (bubbling will occur).

4.2.6 Cover the insulated bucket with the lid and a towel and allow to sit for 10 or more min.

4.2.6 When the liquid nitrogen stops bubbling the irons are ready for use.

4.2.7 The liquid in the bucket may have to be refreshed during cooling depending on the dolphin processing; the level of the liquid should remain at least one inch above the face(s) of the irons.

4.3 Freeze branding

4.3.1 Dolphins can be branded while on the Dolphin Processing Boat or while in the water.

4.3.2 Explain branding process to dolphin handlers and what will be expected of them; verify the identity of the dolphin to be branded (with Data Manager).

4.3.3 Have the insulated bucket, containing the branding irons, brought near to where the branding will take place (if on the Dolphin Processing Boat, ensure that liquid nitrogen will not be able to reach the dolphin or other personnel in the event of a spill).

4.3.4 Organize personnel to fill the various roles: assistant, timer, handlers and photographer.

4.3.5 Freeze brander and assistant should wear cryogloves.

4.3.6 The freeze brander should dry the side of the dorsal fin using a hand towel (ensure that handlers do not allow the fin to become wet after drying).

4.3.7 Make certain that all participants are prepared.

4.3.8 The assistant hands the iron to the brander and the brander selects where on the dorsal fin to place the iron (taking care that there will be sufficient space on the fin to fit all three characters).

4.3.9 Press the face of the brand firmly to the side of the dorsal fin and tell the timer “brand on”.

4.3.10 Apply the iron using firm pressure while alternating a slight side-to-side and up-and-down rocking motion. Don’t allow the face of the iron to lose contact with the skin.

4.3.11 The timer monitors the stopwatch to ensure proper application time (15-20 sec) and should verbally call out the last 5 sec of the application (“5, 4, 3, 2, 1, off”).

4.3.12 Remove brand from skin, photographer quickly takes a close-up picture of the branded area.

4.3.13 Return brand to the assistant who in turn replaces the brand to the 5 gal insulated bucket.

4.3.14 Upon hearing the cue from the photographer, the handlers should re-warm the branded area either by manually palpating the branded area while the dorsal fin is submerged or by firmly applying spongefuls of water directly onto the branded area.

4.3.15 The freeze brander determines when the area has been warmed sufficiently.

4.3.16 Repeat process (beginning at 4.3.6. until both sides of the dorsal fin have been branded with the three character brand.

4.4 Freeze brand clean-up

4.4.1 Return insulated bucket and irons to area where liquid nitrogen was decanted.

4.4.2 Remove brands from bucket and lay them on a towel to re-warm and dry; when dry return to freeze brand kit.

4.4.3 Any liquid nitrogen remaining in the insulated bucket can be decanted back into the Dewar (again taking care to avoid splashing others) or poured out into the water (being careful to make sure no one is nearby).

4.5 Restocking

4.5.1 Update freeze brand order matrix.

4.5.2 Inspect the level of liquid nitrogen in the Dewar and replenish as necessary (between dolphins and at end of work day).

5.0 NUMBERING SYSTEM

Each dolphin was freeze branded using a unique, three-character identifier on the left and right side of the dorsal fin. Charleston dolphins received 800 series (800-899) freeze brands, while IRL dolphins received 900 series (900-999) freeze brands. Even numbers were assigned to males while females received odd numbers. The 800 and 900 series were sequentially applied in both areas. A list of freeze brand identifiers to be used after completing the first 100 numbers at each site are listed below.

5.1 Post-800 freeze brand identifiers for Charleston, SC and post-900 freeze brand identifiers for Indian River Lagoon, FL

Charleston Freeze Brand No. - Male	Charleston Freeze Brand No. - Female	Indian River Lagoon Freeze Brand No. - Male	Indian River Lagoon Freeze Brand No. - Female
8A0	8A1	9A0	9A1
8A2	8A3	9A2	9A3
8A4	8A5	9A4	9A5

Charleston Freeze Brand No. - Male	Charleston Freeze Brand No. - Female	Indian River Lagoon Freeze Brand No. - Male	Indian River Lagoon Freeze Brand No. - Female
8A6	8A7	9A6	9A7
8A8	8A9	9A8	9A9
8F0	8F1	9C0	9C1
8F2	8F3	9C2	9C3
8F4	8F5	9C4	9C5
8F6	8F7	9C6	9C7
8F8	8F9	9C8	9C9
8J0	8J1	9D0	9D1
8J2	8J3	9D2	9D3
8J4	8J5	9D4	9D5
8J6	8J7	9D6	9D7
8J8	8J9	9D8	9D9
8K0	8K1	9V0	9V1
8K2	8K3	9V2	9V3
8K4	8K5	9V4	9V5
8K6	8K7	9V6	9V7
8K8	8K9	9V8	9V9
8M0	8M1	90A	91A
8M2	8M3	92A	93A
8M4	8M5	94A	95A
8M6	8M7	96A	97A
8M8	8M9	98A	99A
8N0	8N1	90C	91C
8N2	8N3	92C	93C
8N4	8N5	94C	95C
8N6	8N7	96C	97C
8N8	8N9	98C	99C
8R0	8R1	90D	91D
8R2	8R3	92D	93D
8R4	8R5	94D	95D
8R6	8R7	96D	97D
8R8	8R9	98D	99D
8T0	8T1	90V	91V
8T2	8T3	92V	93V
8T4	8T5	94V	95V
8T6	8T7	96V	97V
8T8	8T9	98V	99V
8V0	8V1	9X0	9X1
8V2	8V3	9X2	9X3
8V4	8V5	9X4	9X5
8V6	8V7	9X6	9X7
8V8	8V9	9X8	9X9
8W0	8W1	90X	91X
8W2	8W3	92X	93X
8W4	8W5	94X	95X
8W6	8W7	96X	97X
8W8	8W9	98X	99X
8X0	8X1		
8X2	8X3		
8X4	8X5		

Charleston Freeze Brand No. - Male	Charleston Freeze Brand No. - Female	Indian River Lagoon Freeze Brand No. - Male	Indian River Lagoon Freeze Brand No. - Female
8X6	8X7		
8X8	8X9		
8Y0	8Y1		
8Y2	8Y3		
8Y4	8Y5		
8Y6	8Y7		
8Y8	8Y9		
80A	81A		
82A	83A		
84A	85A		
86A	87A		
88A	89A		
80F	81F		
82F	83F		
84F	85F		

6.0 REFERENCES

Anon. A guide to freeze branding. Stone Manufacturing & Supply Co., 1212 Kansas Ave., Kansas City, MO. 64127. 4 pp.

APPENDIX F

Bottlenose Dolphin Rototag Application Procedure

1.0 OBJECTIVE

Describe procedure for rototagging bottlenose dolphins.

2.0 PERSONNEL/TRAINING/RESPONSIBLITES:

Only personnel that have been trained in these techniques should perform these procedures. Only veterinarians should perform invasive procedures.

3.0 MATERIALS

(2) Miltex N-Tralig Intraligamentary syringe (*i.e.*, injector gun)
(~50) Lidocaine HCl 2% and epinephrine ampules
(~20) 30-gauge extra-short dental needles
(10 each) Nasco blue and pink jumbo rototags
(2) Nasco jumbo rototag applicators
(2 sets) #1 and #2 cork borer
(1) cork borer sharpener
(1) 3M cork borer stop
(1) small Rubbermaid container containing gauze soaked in chlorhexiderm
(1) small Rubbermaid container containing gauze soaked in isopropyl alcohol
(~50) alcohol prep pads
(~20) DMSO vials
(1 sheet) extra fine sandpaper
(1) hemostat
(1) black Sharpie
(1) dorsal fin board
(1) Canon EOS1D 35mm digital camera

4.0 GUIDELINES

4.1 Rototag application

4.1.1 Select a site for tag(s) application near the trailing edge of the dorsal fin. First examine the fin in order to avoid major blood vessels and consult with photo-ID staff (females receive pink tag(s) typically high on fin whereas males receive blue tag(s) usually low on fin).

4.1.2 Using the #2 cork borer measure two diameters in from the trailing edge and mark location with a black Sharpie.

4.1.3 Swab the selected site using chlorhexiderm gauze (ensure both sides of fin are cleaned).

4.1.4 Swab the selected site using isopropyl alcohol gauze (ensure both side of fin are disinfected). Once cleaned, avoid touching the selected location.

4.1.5 Inject the lidocaine solution using the injector gun fitted with a 30-gauge needle around the selected site (approximately 5-6 single click injections). Take care to avoid penetrating through the dorsal fin.

4.1.6 Bore a hole through the site by applying steady pressure combined with a 'twisting' motion with the cork borer and with the corkborer stop held firmly against the opposite side of the fin.

4.1.7 Remove tissue plug from cork borer and place in DMSO vial (labeled with dolphin freeze brand number); take care to handle tissue plug with either hemostats or gloved hands.

4.1.8 There should be little to no bleeding; if excess bleeding does occur, stop the bleeding using direct pressure, an icepack or ferric subsulfate-soaked gauze, prior to applying the tag.

4.1.9 Clip the tag through the bored hole using the rototag pliers.

4.1.10 Record the rototags(s) number and location on fin and take pictures of both sides of the dorsal fin (using the dorsal fin board as a backdrop).

4.2 Cleaning rototag equipment and restocking

4.2.1 Dispose of dental needle(s) from the injector gun in a sharps container (after each dolphin).

4.2.2 Inspect #2 cork borer and sharpen if necessary (after each dolphin).

4.2.3 Clean #2 cork borer and rototag pliers with isopropyl gauze (after each dolphin).

4.2.4 Inspect rototag kit and restock as necessary (at end of work day).

APPENDIX G

Date:	Set:

Set Information

Survey #: _____ **Sighting #:** _____ **Net Out Time:** _____

Capture Location: _____

Lat: _____ **Lon:** _____

Water Temperature: _____ °C

Salinity: _____ ppt

Water Depth: _____ m

 # Encircled: _____ **# Escaped:** _____ **# Set Free:** _____

 # Calves: _____

Animal ID's: _____

Comments:

_____

_____

_____

_____

_____

_____

_____

_____

Bottlenose Dolphin HERA Project *Modified 04/2005*

Blood Tubes*:	FB:	Date:	Set:

*color and number (e.g. Blue1)

Captured Individual

Sex (circle one): M F

Time Hit Net: _____ **Time Back in Water:** _____

Time Out of Water: _____ **Time Released:** _____

☐ **Venipuncture (pre only):**
Needle In: _____ *Needle Out:* _____

☐ **Temperature**

☐ **Morphometrics**

☐ **Photos**

☐ **Physical Exam**

☐ **Skin Assessment**

☐ **Cultures:**
☐ *Blowhole* ☐ *Fecal* ☐ *Gastric* ☐ *Lesion(s)*

☐ **Urine** _____ *ml*

☐ **Feces**

☐ **Milk** _____ *ml*

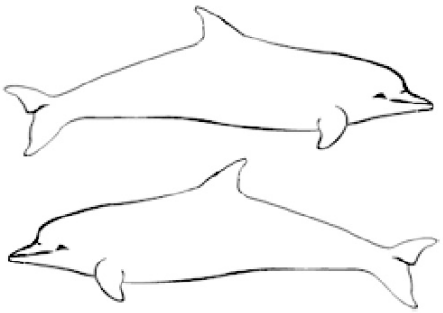
☐ **Tooth Extracted:**
Injection: _____ *Extracted:* _____
Quantity _____
Tooth #: _____ *Left* *Right*

☐ **Blubber Biopsy:**
Injection: _____ *Extracted:* _____

☐ **Skin Lesion Biopsy** *Quantity:* _____

☐ **Freezebrand:**
Start: _____ *End:* _____
Left:
___*sec* # ___*sec* # ___*sec*
Right:
___*sec* # ___*sec* # ___*sec*

Sketch scars, marks, tags, freezebrands, etc:



Comments (treatments, behavior, etc.):

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Roto Tag(s): ☐ Yes ☐ No	**Radio Tag:** ☐ Yes ☐ No	**Satellite Tag:** ☐ Yes ☐ No
Quantity: _____	Frequency: _____	ID: _____
Site Prep: ☐ Punch ☐ Awl/Drill ☐ Other _____	Site Prep: ☐ Punch ☐ Awl/Drill ☐ Other _____	Site Prep: ☐ Punch ☐ Awl/Drill ☐ Other _____
Number(s): _____	Exp Life: _____days	Exp Life: _____days
Location(s): _____	Weight: _____g	Duty Cycle: _____
Color(s): _____	Mfg: _____	Mfg: _____
	Pulse Rate/Width: _____/_____	
	Attachment: ☐ Bullet ☐ Roto/Radio ☐ Other _____	

FB:	Date:	Set:

Physical Exam

Veterinarian: _____

1. Behavioral Assessment/Critical Care Observations (*during processing):

Respirations: ☐ Full ☐ Shallow
Respiratory Interval: ☐ Prolonged ☐ Adequate ☐ Rapid
Respiratory Effectiveness*: ☐ Improved ☐ Degraded ☐ Uniform
Eye Appearance: ☐ Bright (Responsive) ☐ Dull
Mucus Membrane Color: ☐ Pink ☐ Pale ☐ Cyanotic
Mucus Membrane CRT: _____ sec
Whistling/Vocalizing: ☐ Yes ☐ No
W/V Frequency*: ☐ Increased ☐ Decreased ☐ Uniform
Spontaneous Vomiting: ☐ Yes ☐ No
Arching: ☐ None ☐ Head Only ☐ Fluke Only ☐ Head and Fluke
Assessment Summary*: _____

_____

_____

2. Body condition index (1-5):

☐ Emaciated (1) ☐ Underweight (2) ☐ Ideal (3) ☐ Overweight (4) ☐ Obese (5)

3. Post-nuchal fat pad (1-4):

☐ Concave (1) ☐ Spongy (2) ☐ Firm (3) ☐ Convex (4)

4. Oral cavity: ☐ **WNL** ☐ **Abnormal**

If abnormal, describe findings:

_____

_____

_____

Gingival Hyperplasia: ☐ Yes ☐ No ☐ Mild ☐ Moderate ☐ Severe
Upper Left Tooth Wear: ☐ None ☐ Few ☐ Moderate ☐ Excessive
Lower Left Tooth Wear: ☐ None ☐ Few ☐ Moderate ☐ Excessive
Upper Right Tooth Wear: ☐ None ☐ Few ☐ Moderate ☐ Excessive
Lower Right Tooth Wear: ☐ None ☐ Few ☐ Moderate ☐ Excessive
Comments: _____

_____

Modified 05/2005

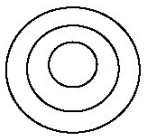
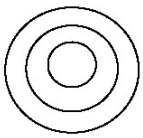
**see Appendix H for detailed body condition index descriptions*

FB:	Date:	Set:

5. Eyes: ☐ **WNL** ☐ **Abnormal**

Describe and Diagram: _____

_____

Right:  Left: 

6. Cardiovascular: ☐ **WNL** ☐ **Abnormal**

Rate (/min): Pre-resp: _____ Post-resp: _____

Rhythm: ☐ Regular Sinus Arrhythmia ☐ Abnormal Arrhythmia

Abnormal Sounds: ☐ Yes ☐ No

If abnormal sounds (murmurs, *etc.*) are observed, describe and grade: _____

_____

7. Respiratory System: ☐ **WNL** ☐ **Abnormal**

Rate (over one minute) _____

Abnormalities: ☐ Rales ☐ Wheezes

Blow odor: ☐ None ☐ Normal ☐ Malodorous

Mucus: ☐ None ☐ Mild ☐ Moderate ☐ Severe

8. GI Tract: ☐ **WNL** ☐ **Abnormal**

Gut sounds ☐ Present ☐ Not Present

Gastric fluid ☐ WNL ☐ Abnormal pH _____

Feces ☐ WNL ☐ Abnormal

If abnormal, describe texture, color, odor, *etc.*: _____

_____

9. Reproductive:

Genital slit ☐ WNL ☐ Abnormal

Vagina/Penis ☐ WNL ☐ Abnormal

Right Mammary ☐ WNL ☐ Abnormal

Left Mammary ☐ WNL ☐ Abnormal

Describe abnormalities: _____

_____

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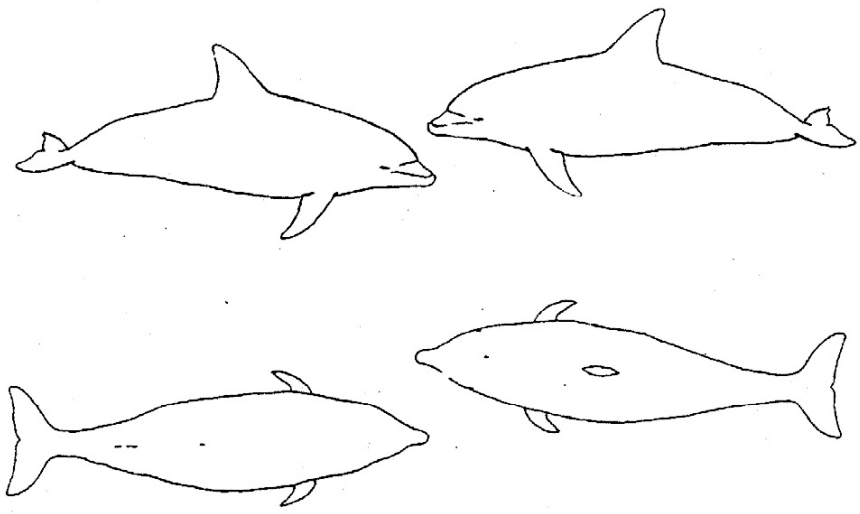
FB:	Date:	Set:

Skin Assessment

Epidermal Sloughing: ☐ None ☐ Generalized ☐ Focused

Rake Marks: ☐ 0 ☐ 1-2 ☐ 3-5 ☐ >5

Indicate location(s) of lesions described using corresponding Lesion(s) Description # :



Comments:

FB:	Date:	Set:

Lesion Descriptions

Lesion(s) Description #: _____

Distribution: ☐ Single Lesion ☐ Multiple Lesions

Type: ☐ Fishery Int. ☐ Predator ☐ Other Traumatic
☐ Lobomycosis ☐ Papilloma ☐ Pox ☐ Other Infectious

Status: ☐ Active ☐ Inactive ☐ Active/Healing ☐ Healed ☐ NA

Color(s): ☐ Black ☐ Gray ☐ White ☐ Red ☐ Other _____

Shape(s): ☐ Pinhole ☐ Round ☐ Irregular ☐ Tattoo ☐ Other _____

Site(s): ☐ Dorsal ☐ Lateral ☐ Ventral

Form(s): ☐ Flat ☐ Raised ☐ Depressed ☐ Ulcerated ☐ Cauliflower
☐ Other _____

Biopsy: ☐ Frozen ☐ Histopath ☐ None

Single Lesion Only:

Size: ☐ < 1 cm^2 ☐ 1-3 cm^2 ☐ > 3 cm^2

Consistency: ☐ Firm ☐ Soft ☐ Gelatinous ☐ Other _____

Multiple Lesions Only:

Pattern: ☐ Focal ☐ Multifocal ☐ Multifocal to Coalescing ☐ Diffuse ☐ Other

Severity: ☐ Mild (≤10%) ☐ Moderate (11-50%) ☐ Severe (>50%) ☐ NA

Lesion(s) Description #: _____

Distribution: ☐ Single Lesion ☐ Multiple Lesions

Type: ☐ Fishery Int. ☐ Predator ☐ Other Traumatic
☐ Lobomycosis ☐ Papilloma ☐ Pox ☐ Other Infectious

Status: ☐ Active ☐ Inactive ☐ Active/Healing ☐ Healed ☐ NA

Color(s): ☐ Black ☐ Gray ☐ White ☐ Red ☐ Other _____

Shape(s): ☐ Pinhole ☐ Round ☐ Irregular ☐ Tattoo ☐ Other _____

Site(s): ☐ Dorsal ☐ Lateral ☐ Ventral

Form(s): ☐ Flat ☐ Raised ☐ Depressed ☐ Ulcerated ☐ Cauliflower
☐ Other _____

Biopsy: ☐ Frozen ☐ Histopath ☐ None

Single Lesion Only:

Size: ☐ < 1 cm^2 ☐ 1-3 cm^2 ☐ > 3 cm^2

Consistency: ☐ Firm ☐ Soft ☐ Gelatinous ☐ Other _____

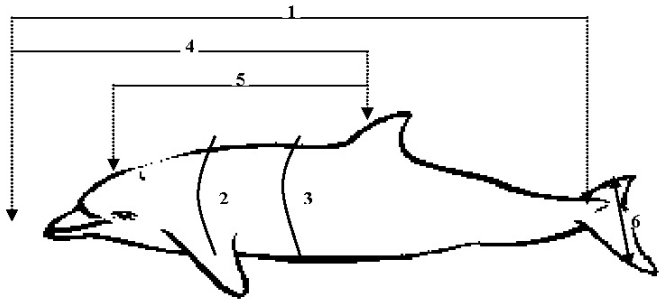
Multiple Lesions Only:

Pattern: ☐ Focal ☐ Multifocal ☐ Multifocal to Coalescing ☐ Diffuse ☐ Other

Severity: ☐ Mild (≤10%) ☐ Moderate (11-50%) ☐ Severe (>50%) ☐ NA

FB:	Date:	Set:

Morphometrics



Total Length (1): _____ **cm** - straight line measurement, parallel to the body, from the tip of the rostrum (upper jaw) to the fluke notch.

Axillary Girth (2): _____ **cm** - circumferential measurement of the torso immediately caudal to the pectoral fins.

Maximum Girth (3): _____ **cm** -circumferential measurement of the torso immediately cranial to the origin of the dorsal fin.

Tip of Rostrum to Tip of Dorsal Fin (4): _____ **cm** -straight line measurement, parallel to the body, from the tip of the rostrum (upper jaw) to the distal tip of the dorsal fin.

Blowhole to Tip of Dorsal Fin (5): _____ **cm** - straight line measurement, parallel to the body, from the center of the blowhole to the distal tip of the dorsal fin.

Fluke width (6): _____ **cm** - straight line fluke width from left to right distal tip.

Weight (including sling): _____ **lbs**

Length of Right Testicle: _____ **cm** (*from ultrasonographic exam*)

Length of Left Testicle: _____ **cm** (*from ultrasonographic exam*)

Upper Left Tooth Count: _____ **Upper Right Tooth Count:** _____

Lower Left Tooth Count: _____ **Lower Right Tooth Count:** _____

Comments:

_____

_____

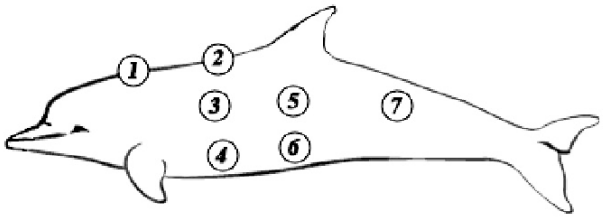
_____

_____

Bottlenose Dolphin HERA Project *Modified 04/2005*

FB:	Date:	Set:

Ultrasonographic Exam



Site	Description	Blubber Depth Measurements
1	Post-nuchal tissue (full thickness)	**Including Blubber:** _____ **cm** **Excluding Blubber:** _____ **cm**
2	10cm cranial to dorsal fin leading edge	**Int**: _____ **cm** **Ext**: _____ **cm**
3	Mid lateral, 10cm cranial to dorsal fin leading edge	**Int**: _____ **cm** **Ext**: _____ **cm**
4	Ventral mid line, 10cm cranial to dorsal fin leading edge	**Int**: _____ **cm** **Ext**: _____ **cm**
5	Mid lateral, midpoint of dorsal fin	**Int**: _____ **cm** **Ext**: _____ **cm**
6	Ventral mid line, midpoint of dorsal fin	**Int**: _____ **cm** **Ext**: _____ **cm**
7	Blubber biopsy site	**Int**: _____ **cm** **Ext**: _____ **cm**

Liver:	☐ Non-remarkable	☐ Abnormal *(note below)*
Urinary Tract:	☐ Non-remarkable	☐ Abnormal (*note below*)
Reproductive Tract:	☐ Non-remarkable	☐ Abnormal (*note below*)
Cardiovascular:	☐ Non-remarkable	☐ Abnormal (*note below*)
Stomach:	☐ Non-remarkable	☐ Abnormal (*note below*)

Comments (Abnormal Findings, Tentative Diagnoses, etc.):

_____
_____
_____
_____
_____
_____
_____
_____

Ultrasonographer: _____ **Signature:** _____

Modified 06/2005

FB:	Date:	Set:

Temperature

Observer: _____

Time	Temperature (°C)			Start & End of Procedures/Comments
	Dorsal Fin	Melon	Flukes	

*** Record temperatures every 5 minutes**

FB:	Date:	Set:

Respiration Rate

Observer: _____

NOTIFY VETERINARY STAFF IF:

- Animal holds breath one minute or longer.
- Respiration rate is greater than 8 breaths/minute.
- Breathing sounds "unusual" (not full, deep, smooth) or is "partial" (i.e., exhalation only)

Time of Respiration			Start & End of All Procedures/Comments
Hour	Min.	Sec.	

Bottlenose Dolphin HERA Project *Modified 04/2005*

Blood Tubes*:	FB:	Date:	Set:

*color and number (e.g. Blue1)

Sample Processing

ON-SITE TESTS

URINE Test Strip:

Test Parameter	
specific gravity	
pH	
leukocytes	
Nitrite	
Protein (mg/dL)	
Glucose (mg/dL)	
ketones	
Urobilinogen (mg/dL)	
Billirubin	
Blood (Ery/uL)	
Hemoglobin (Ery/uL)	

ESR:

Start Time	
End Time	
Results	
Analyst	

I-STAT:

I-Stat ID (rep 1)	
I-Stat ID (rep 2)	
I-Stat ID (rep 2)	
Analyst	

Gastric pH:

pH	

Microalbumin:

Negative	
Low Positive	
Medium Positive	
High Positive	
Very High Positive	
Invalid – list reason	

SAMPLE CHECKLIST

☐ **VENIPUNCTURE**
Note tubes missing _____
Note any hemolyzed tubes _____
Replicate Lab Tests, if Yes note tube ID _____

☐ **CULTURES**
__ Blowhole, __ Fecal, __ Gastric, __ Lesions

☐ **CYTOLOGY**
__ Blowhole, __ Fecal, __ Gastric, __ Lesions

☐ **URINE**, Total Vol._____ml

☐ **FECES**

☐ **TOOTH**

☐ **BIOPSY**
Blubber- Full depth __ Yes __ No

☐ **SKIN LESION** __ Frozen __ Formalin

☐ **PARASITES**

Comments (note samples not collected/tests not done):

Water/Sediment Sampling Data Sheet

Date: _____

Set: _____

Collector: _____

***Place label on each sample container with Date and Set Number*

YSI Readings

Water Temperature: _____ ° C
Salinity: _____ ppt

Time of water/sediment collection: _____

GPS waypoint of water/sediment collection: _____

Latitude of water/sediment collection: _____

Longitude of water/sediment collection: _____

Water depth at collection site: _____

Notes:

(i.e. strong currents, wind, developed vs. undeveloped shoreline, mangroves, high boat traffic, flocks of birds/wildlife present, and other environmental variables at collectors discretion)

_____

_____

_____

_____

_____

_____

APPENDIX H

Score	Body Condition Description
1	**Emaciated Body Condition –** Concavity of epaxial muscles. Severe overt post-nuchal depression (*i.e.*, peanut-shaped head) and palpable minimal post-nuchal fat turgidity; bony structures such as ribs and peduncular vertebrae visually evident. Integument freely slides over underlying muscle.
2	**Underweight Body Condition –** Slight concavity to epaxial muscles. Slight to moderate overt post-nuchal depression (*i.e.*, +/- peanut-shaped head). Palpable decreased post-nuchal fat turgidity. Ribs and peduncular vertebrae only visually evident with positional changes of body.
3	**Ideal Body Condition –** No concavity of epaxial surfaces and post-nuchal fat pad. Palpable moderate post-nuchal fat turgidity. Ribs and peduncular vertebrae not visually evident. Sleek body contours.
4	**Overweight Body Condition –** No concavity of epaxial surfaces and post-nuchal fat pad. Firm palpable post-nuchal fat pad turgidity. Small amount of rolls of fatty tissue around neck. Dorsal epaxial mild convexity. Slight dorsal epaxial convexity with slight depression of dorsal midline.
5	**Obese Body Condition –** No concavity of epaxial surfaces and post-nuchal fat pad. Very firm palpable post-nuchal fat pad. Overt multiple rolls of fatty tissue around neck. Eyes deeply set within orbits. Dorsal epaxial convexity with depression of dorsal midline.