Guidance to Parties Interested in Conducting Biopsies on Cook Inlet Beluga Whales

Prepared by the National Marine Fisheries Service, Alaska Region (NMFS AKR)
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The topic of conducting biopsy studies on endangered Cook Inlet beluga whales is a recurring one. In an effort to provide expert-generated guidance for undertaking such studies, NMFS AKR sponsored a Cook Inlet beluga whale biopsy workshop in April 2014. Participants brought to the table experience with biopsy methods, tissue collection procedures, lab analyses of biopsy samples, as well as an appreciation of the Endangered Species Act. The workshop report is available on the NMFS AKR webpage[1]. NMFS AKR's views regarding funding, permitting, or authorizing biopsy studies of Cook Inlet beluga whales will be informed by the contents of this report. The agency urges those developing proposals that include obtaining biopsy samples from Cook Inlet beluga whales to incorporate the recommendations from this report into their study plans.

In addition to the workshop conclusions and recommendations, NMFS AKR provides the following additional guidance for working with this endangered species:

1) Biopsy methodology should not be *developed* using endangered Cook Inlet beluga whales as subjects. Investigators seeking to develop beluga whale biopsy methodology should target non-listed beluga populations. Methods should be developed elsewhere (on other beluga whales or small cetaceans) and shown to be effective.

2) Investigators using established methods who seek to address research questions using biopsy samples from Cook Inlet beluga whales should develop a study plan that demonstrates a clear link between the research question and a practical management application. In other words, investigators should explain how their work will inform future actions to conserve and recover this population.

3) If a research question can be addressed using multiple methodologies, the least invasive methodology should be used. The decision to use a more invasive methodology must be well justified, and the management-related advantages of using the more invasive methodology must, in the opinion of NMFS AKR, outweigh the additional risk imposed upon the whales.

4) Investigators should take steps to associate each biopsy sample to a known individual.
   a) Investigators should collect photo identification-quality images of the whales to be biopsied prior to attempting to obtain a biopsy sample.
   b) Investigators must attempt to obtain photo documentation of the location of the biopsy site on the sampled animal to aid in future photo identification efforts of that individual. Images will be publicly available and will be incorporated into the Cook Inlet Beluga Whale Photo Identification database.
   c) Investigators should adopt existing Photographic ID protocols developed by investigators with the Cook Inlet Beluga Whale Photo ID project.

[1] http://alaskafisheries.noaa.gov/protectedresources/whales/beluga/workshop/cibbiopsyworkshop0614.pdf

5) Tethered darts/bolts have been used successfully for remote biopsy of beluga whales elsewhere, and should be seriously considered for use in Cook Inlet. Doing so will likely be less disruptive to the beluga whales and may result in retrieval of a greater number of samples than could be obtained from disruptive watercraft operating in proximity to Cook Inlet beluga whales while attempting to retrieve untethered bolts/darts before they sink.

6) Whenever it is feasible to conduct remote biopsy sampling of Cook Inlet beluga whales from shore, NMFS AKR encourages shore-based remote biopsy sampling instead of boat-based sampling as a method to reduce whale disturbance.

7) When investigators determine that watercraft will be used for collection of biopsy samples, watercraft should be operated in a manner that avoids harassment of, or harm to, Cook Inlet beluga whales.
   a) Investigators must not engage in chasing, herding, or capturing Cook Inlet beluga whales to obtain samples.
   b) To reduce the risk of propeller-induced injury to whales, remote biopsy darts or bolts should be fired only from an anchored or drifting boat.
   c) Watercraft should be equipped with jet drives or propeller guards throughout the sampling operation.
   d) To reduce the potential for inaccurate shots caused by an unsteady platform, remote biopsy darts or bolts should only be deployed from watercraft in Beaufort sea states of 1 or less.

8) NMFS AKR encourages the selection of shore-based sampling locations that are not likely to draw spectators. NMFS AKR recognizes that boats may be necessary to retrieve samples and that sometimes it may be necessary to conduct sampling at locations that may draw public attention. In situations where spectators may gather, one individual should remain on-site during sampling who will be dedicated to public education and outreach to anyone observing the remote biopsy sampling operations.

9) Biopsy collection methodologies and field protocols should follow national standards developed for cetacean biopsy darting programs.
   a) The National Institute of Standard and Technology (NIST) has developed Cetacean Dart Biopsy Protocols (attached), which included input collected during the Development of Standardized Procedures for Cetacean Dart Biopsy Sampling Workshop held before the 19th Biennial Conference on the Biology of Marine Mammals.
   b) To reduce the risk of analysis error or complications, NIST accredited laboratories should be used for sample analysis.

10) Prior to implementing a Cook Inlet beluga whale biopsy study, a study plan should be prepared that indicates the feasibility of obtaining the required number of samples to adequately address the research question with appropriate accuracy and precision. The maximum number of whales that must be biopsied to obtain reasonable statistical confidence in the results should be explicitly stated. The study plan should also indicate:
   a) How and where samples will be stored, and who will be the custodian of the samples.
   b) When and how ISO 19115/19110-compliant project metadata, data metadata, and QA/QCed data will be archived and made publicly available on the internet (see http://www.ncddc.noaa.gov/metadata-standards/).

11) The collection of the biopsies must be conducted only by qualified individuals with demonstrated proficiency using the deployment device.
    a) The shooter should have taken at least 100 practice shots with the sampling device within one week of sampling operations.
    b) For the final 50 practice shots, the shooter should be able to place 100% of darts/bolts into a 15 cm diameter circle from a 5m distance, and greater than 50% of all darts/ bolts into a 15 cm diameter circle from a 10m distance.
    c) At least 30 practice shots should be at targets moving in water at velocities comparable to that of beluga whales. If the shooter will be operating from a watercraft, these water-based practice shots should occur from the same watercraft to be used during sampling operations, or from a watercraft of similar design. For the final 15 water-based practice shots, the shooter should be able to place 100% of darts/bolts into a 15cm diameter circle from a 5m distance, and greater than 50% of darts/bolts into a 15 cm diameter circle from a 10m distance.
    d) Study proposals, study plans, and permit applications should specify the past experience of the shooter in obtaining remote biopsy samples. The past success of the shooter should be expressed as the number of usable samples obtained per the total number of shots fired at cetaceans.

12) All Cook Inlet beluga whale biopsy programs should include an active outreach component. Public perception and understanding of an activity that involves "shooting" at endangered beluga whales must be taken seriously. Prior to starting any biopsy collection activity, the public should be made aware of the activity with information indicating the study's goals and objectives, and how the activity will benefit the belugas.

ATTACHMENT: NIST CETACEAN BIOPSY PROTOCOLS – FIELD COLLECTION SECTIONS

Cleaning Dart Biopsy Tips

Materials:

- Powder free vinyl gloves
- Micro-test tube brush (must fit into the diameter of the biopsy tip)
- Liqui-Nox soap
- High purity deionized water
- Chlorine bleach (4.0-7.0 % sodium hypochlorite in water) (buy at local store)
- High purity isopropanol in clean PTFE squirt bottle
- Glass beakers (rinse with high purity deionized water then high purity isopropanol to clean before use)
- Forceps (rinse with high purity deionized water then high purity isopropanol to clean before use)
- GC grade hexane rinsed foil (each piece is folded in half so each clean half is touching the other)
- Whirl-Pak bags

Procedure:

- Put on a clean pair of powder free vinyl gloves
- Pour a drop of Liqui-Nox soap into a clean beaker and fill with high purity deionized water
- Scrub and rinse dirty biopsy tips with soapy water and test tube brush to remove all remaining tissue
- Check to make sure that the tip is in good shape: sharp with all 3 prongs present
- Rinse biopsy tips with high purity deionized water and place in another clean beaker
- Cover biopsy tips with 5% chlorine bleach solution made with high purity deionized water and soak for 5 minutes to sterilize tips
- Drain bleach solution from beaker
- Using high purity deionized water, rinse and drain tips in the beaker 3 times
- Individually remove biopsy tips from beaker using pre-cleaned forceps and thoroughly rinse the tip inside and out with isopropanol from the squirt bottle.
- Place tips on hexane rinsed foil to dry
- After tips are dry, using clean gloved hands and pre-cleaned forceps, place the tips individually into Whirl-Pak bags

Cleaning Dart Bolt and Installing Clean Dart Biopsy Tip

Materials:

- High purity deionized water in clean PTFE squirt bottle with PTFE bag over top
- High purity isopropanol in clean PTFE squirt bottle with PTFE bag over top

Procedure:

- Squirt dart bolt threads with high purity deionized water and then isopropanol before screwing on biopsy tip
- Holding the outside of a Whirl-Pak with a clean biopsy tip inside, screw the biopsy tip on to the dart and leave the Whirl-Pak overtop until it is ready to be used

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

1. Hydrocarbons and trace elements from sources other than the animal. These include: sunscreen, boat fuel, engine exhaust, insect repellent, and other oils associated with the sampling platform.
2. Carry-over contamination from the previous animal. The concentrations of organohalide pollutants in cetaceans can vary by nearly two orders of magnitude.

Dart Biopsy Collection in the Field

Materials:

- Fine mesh net
- GC grade hexane rinsed foil (each piece is folded in half so each clean half is touching the other)
- Whirl-Pak bags
- Permanent marker
- High purity isopropanol in solvent cleaned PTFE squirt bottle with PTFE bag over top
- High purity deionized water in clean PTFE squirt bottle with PTFE bag over top

Procedure:

- Before the dart is loaded onto the crossbow, remove the Whirl-Pak that is covering the clean tip (if the dart is not used and has not touched anything the Whirl-Pak bag can be placed back over the tip for future use)
- Once the dart is shot at an animal, collect it from the water with a net making sure that the dart fin end enters the net first and not the biopsy tip with the sample
- After the dart is retrieved, using the inside hexane rinsed side of the aluminum foil, unscrew the biopsy tip from the dart
- Wrap the biopsy tip in hexane rinsed aluminum foil and place back in original Whirl-Pak or use a new clean Whirl-Pak
- Label the outside of the bag with appropriate sample identification information and place in a cooler on ice

Please make a note if there is any modification to the protocol in the field

If a clean biopsy tip touches anything while exposed in preparation for shooting, rinse tip with isopropanol before reuse.

If a dart is shot into the water at an animal and misses, it can be reused once it is rinsed with high purity deionized water and then isopropanol.[2]

If a dart is shot at an animal, hits the animal, and does not retrieve a sample, the biopsy tip cannot be used again until it goes through the full cleaning procedure.

If a dart needs to be reused on the same day for another clean biopsy tip, rinse the dart threads with high purity deionized water and then isopropanol before screwing on a clean biopsy tip.

[2] *NMFS AKR note: NMML protocol requires rinsing with water after isopropyl if the sampling tip is to be re-used immediately.*