

GENERAL SAMPLING PROTOCOLS: BIOTOXIN ANALYSIS & PHYTOPLANKTON IDENTIFICATION

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A. Marine Animal Sample Collection

Supplies

- * Normal sized samples: 50-mL plastic centrifuge tubes or other plastic container
- * Large samples (e.g. whole fish, shrimp, etc.): sealable/zip-lock plastic bags or bottles

** Prior to collection, obtain required permits or licenses in order to comply with state and/or federal regulations for shellfish or protected species (marine mammals, sea turtles).

Invertebrates, (clams, oysters, mussels, scallops, crustaceans)

Generally collect entire animal. Do not store in formalin, ethanol, or other preservative solvents. Freeze bivalves whole or shucked, up to 50 g meat/tissue. Larger organisms can be dissected and organs sampled as necessary. Samples can be stored in ziplock bags on ice until they can be frozen. Freeze (-20°C) and ship on dry ice. Collection of shellfish is most easily accomplished by the use of available harvesting methods (rakes, dredges, etc.)

Prey Fish

If possible, the species should be identified before freezing. Small fish should be collected and frozen, then shipped whole. For large species, stomach contents (whole stomach), liver and flesh should be sampled and stored separately. Minimum of 10 g flesh should be obtained. Do not store in formalin, ethanol, or other preservative solvents. All tissues can be stored frozen (-20°C) in ziplock bags until shipment on dry ice.

Marine Mammals

(also see Geraci, J.R. and Lounsbury, V.J. 2005. Marine Mammals Ashore: A Field Guide for Strandings. National Aquarium, Baltimore, MD, 372 pp. for detailed necropsy sampling procedures). Sampling of code 1 or 2 animals is preferred (see above reference for definition), although code 3 animals and later are still useful for toxin analysis.

The most useful tissues/fluids for confirming biotoxin exposure are generally liver, feces, urine and stomach contents. However, samples from additional compartments (intestinal contents, kidney, lung, brain, whole blood, serum) are also valuable depending on the toxins of interest, and are useful for metabolism and body burden studies. All samples should be immediately placed in a cooler on ice and frozen (-20°C or -80°C) as soon as possible after collection. Do not store in formalin, ethanol, or other preservative solvents. Samples should be shipped on dry ice to the laboratory for analysis. Prior to shipping samples, please contact the receiving laboratory to ensure proper receipt of the samples and sample data.

All sample containers must be labeled with the animal ID and sample type in indelible ink (include date and species if space permits), such that labels remain legible when wet. When this is not possible, a small tag containing sample information inserted inside the sample container may be useful. A copy of the NOAA level A datasheet for each animal must accompany each shipment. If this datasheet is not available, please include the following data with the sample shipment: species and common name, stranding date (typically date of initial observation), stranding location (latitude/longitude in decimal degrees,), animal length, weight, condition code, sex, and any additional relevant information. In addition, also send a digital version of data sheets and sample logs to your contact at the laboratory. Please include alternate animal IDs when multiple field ID numbers exist. Animal IDs should be consistent with those submitted to the national stranding database. Sample containers and **volumes listed below are recommended but not required.**

Urine - Collect a minimum of 1 ml urine, more if available (up to 50 ml). Store frozen (-20°C) in capped plastic centrifuge tubes.

Feces - Collect a minimum of 5 g (up to 50 g). Store frozen (-20°C) in capped plastic centrifuge tubes or other container suitable for freezer storage.

Intestinal contents - Collect a minimum of 5 g (up to 50 g). Store frozen (-20°C) in capped plastic centrifuge tubes or other container suitable for freezer storage. Indicate which portion of the intestine was sampled (e.g. upper, mid-, lower intestine)

Stomach contents - Collect a minimum of 5 g (up to 50 g) of solid or semi-solid contents if available. Store frozen (-20°C) in capped plastic centrifuge tubes or other container suitable for freezer storage. If stomach fluid only is available, collect at least 5 ml in a plastic tube or vial. Indicate which portion of the stomach was sampled if applicable (e.g. pyloric, fundic, etc.). If stomachs contain undigested or partially digested prey or food items, please collect separately from gastric fluid. Any indication of prey species or identification of contents are very valuable to interpretation of analyses.

Liver, kidney, lung, spleen, brain - collect a minimum of 5 g (up to 50 g). Store frozen (-20°C) in plastic tubes, ziploc bags or other leak-proof containers.

Serum - obtain serum by centrifugation (1500-3000 x g; 5 minutes) of whole, heparinized blood. The top layer is the serum. Collect >0.5 ml of serum and store frozen (-20°C) in a plastic tube.

Whole blood - Heparinized whole blood can be spotted directly onto blood collection cards and stored at room temperature in the presence of desiccant pouches. Blood cards with detailed instructions can be obtained from your contact at the Marine Biotoxins Program laboratory. If blood cards are not available, liquid whole blood may still be useful.

****Please note, if samples are to be analyzed for multiple algal toxins, a larger amount of***

sample may be needed in order to perform multiple toxin extractions.

Birds, Turtles, other species of interest

Collect as above for mammals, however other sample types (e.g. cloacal contents, gizzard contents) may be useful when collection of feces, urine, etc. is not possible.

NOTE: Minimum and maximum animal sample volumes/weights are general guidelines; depending on the geographic region of strandings, additional material may be required in order to split samples for analysis of multiple toxin classes. Additionally, insufficient sample volume should not be a deterrent for collection, particularly in the case of rare events or limited animal availability. Contact project PI directly for more information.

B. Seawater Sample Collection

Supplies

- * Bucket
- * Plastic bottles (100 ml and 1 liter)
- * Plankton net (10 μ m nylon mesh) if available
- * Lugol's iodine fixative
- or
- * glutaraldehyde fixative

Lugol's iodine: - dissolve 10 g potassium iodide (KI) in 100 ml distilled water
 - add 5 g crystalline iodine (I₂)
 - add 10 ml glacial acetic acid

Protocol

1. Look for discolored water patches (record observations). *There may not be discolored water in association with some toxic algal events.* Record date and location (lat/long), and temperature, salinity, and dissolved oxygen if possible.
2. For quantitative toxin/species analysis, collect surface water using bucket. Transfer up to 1 L of water into a capped plastic sampling bottle or other suitable container (rinsed soda bottles are acceptable). Store in a cool, dark place (do not freeze). Ship overnight if possible in styrofoam cooler containing wet paper towels and refrigerated blue ice packs (keep these from actually touching the bottle).
3. For qualitative analysis (if plankton net is available): Collect water sample with a plankton net, concentrating the seawater using a vertical tow (pull from depth to surface until plankton begin to settle at bottom of collection cup). Transfer ~100 ml of concentrated sample to a plastic sampling bottle or other suitable container (rinsed soda bottles are acceptable). Add preservative, if available; add 1-2 ml Lugol's iodine or

Utermohl's solution to make a tea color, or 2 ml glutaraldehyde to make 2% final concentration. Store and ship as above.

C. Shipping Address

Spencer Fire
Florida Institute of Technology (OLS130)
150 W. University Blvd.
Melbourne, FL 32901
321.674.7138

- Please ensure samples will arrive Monday-Thursday in case of any shipping delays.
- Ship Overnight AM Delivery when possible, and forward tracking #s by email.
- Please include sample submission form with shipments.

link to [Current FIT Sample Submission Sheet](#)