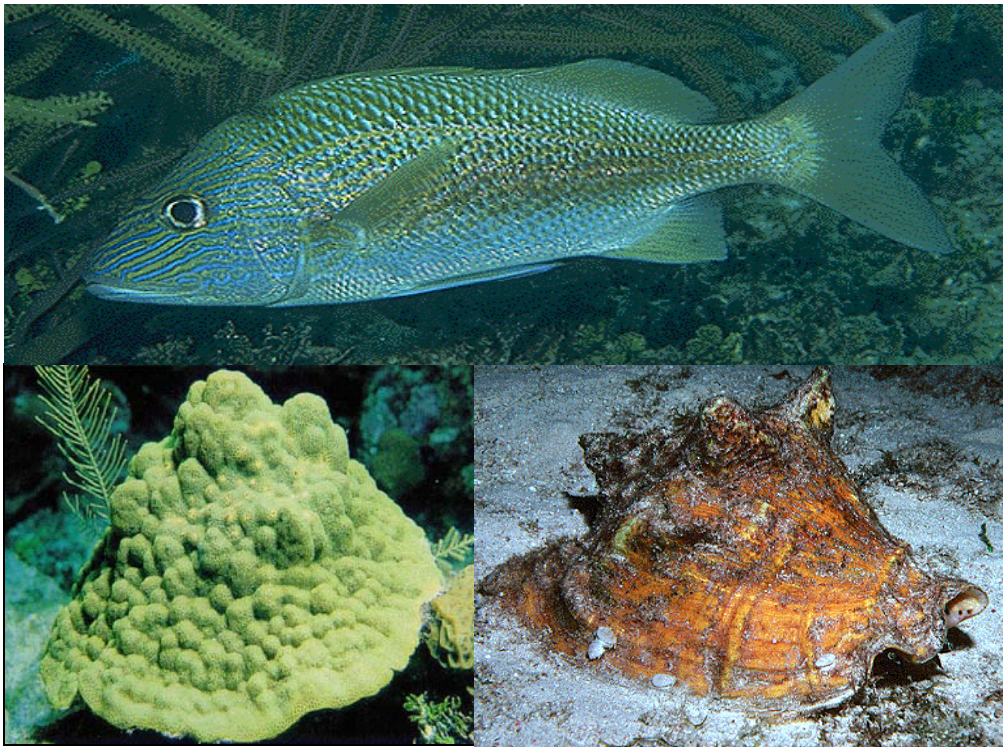
# Contaminant Monitoring Protocol

## Assessing the Effectiveness of Agricultural Better Management Practices in the Mesoamerican Reef



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Haereticus Environmental Laboratory

WWF

ICRAN International Coral Reef Action Network

UNEP

## PREFACE

While excess nutrients and over-fishing are widely recognized as major contributors to global coral reef decline, the role of chemical pollutants, including those derived from agricultural activities, as potential agents of reef ecosystem decline has been less studied or recognized as a significant threat.

However, recent studies within the largely offshore reef habitats of the Mesoamerican Reef of bioaccumulation of agrochemicals in a variety coral reef species have found that such contamination is present on offshore reefs. Our strategy, therefore, involves linking monitoring of bioaccumulation in marine organisms with the agrochemicals used by key industries in the agribusiness sector and our collaborative efforts to reduce those with the highest risks to marine life. The development of this sampling protocol is in an effort to instill a level of rigor and confidence in establishing baseline conditions for subsequent monitoring in the success of these management interventions (better management practices or BMP's) which are aimed at reducing the contamination. It is the first in a series of protocols, and will be followed by protocols for the incorporation of targeted biomarkers and eco-toxicology assays. Together these will help to establish ecological risk framework appropriate to this marine ecosystem.

We encourage collaborations with other researchers from academia, conservation organizations, government and industry from the region and the global community, who have similar aims and monitoring needs.

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## Contaminant Monitoring Protocol for Assessing the Effectiveness of Agricultural Better Management Practices in the Mesoamerican Reef

This document contains contaminant chemistry analysis protocols for organochlorine pesticides and polychlorinated biphenyls, including the collection and analysis of biological and sediment samples. It was prepared for WWF and the ICRAN MAR Alliance by Craig Downs, Executive Director of Haereticus Environmental Laboratory in collaboration with Melanie McField, WWF Centrral America.

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### 1.1.1 Materials

Materials that are needed to collect *Porites asteroides*(coral), *Strombus gigas* (queen conch, mollusc), and *Haemulon plumieri* (white grunt, fish) for organic contaminant chemistry analysis. Units are suggested for a single sampling time-point for 4 sites

| | Item | Source | Catalog # | Units |
|---|---|---|---|---|
| 1. | Sediment Jars, 500 mL | Fisher Scientific | 05-719-521D | 24 |
| 2. | Oak Ridge Centrifuge tube, 50 mL | Nalgene Labware | 3114-0050 | 8 |
| 3. | Oak Ridge Centrifuge tube, 30 mL | Nalgene Labware | 3114-0030 | 60 |
| 4. | Oak Ridge Centrifuge tube, 10 mL | Nalgene Labware | 3114-0010 | 60 |
| 5. | Al Stohlman Brand Drive Punch 13/64” | www.sbeartradingpost.com | 35005-06 | 10 |
| 6. | Stainless steel dissection probe | Fisher Scientific | NC9727105 | 10 |
| 7. | Stainless steel scissors | www.discountofficesupplies.com | | 1 |
| 8. | Surgical scissors | www.drinstruments.com | 6SS | 12 |
| 9. | Stainless steel cutlery knife | | | 1 |
| 10. | Heavy duty aluminum Foil, clean | VWR | 29952-200 | 1 |
| 11. | Hammer | ? | ? | 2 |
| 12. | Chisel, 5”(125) long x 5/16”(8.0) | www.steritool.com | 10280 | 8 |
| 13. | Scalpel, stainless steel | VWR | BD-371030 | 15 |
| 14. | Scalpel blades, stainless steel | VWR | BD-371111 | 1 case |
| 15. | Forceps | VWR | 25716-002 | 12 |
| 16. | Forceps #2 | VWR | 25728-001 | 5 |
| 17. | Teflon Sheets 12’x24’ ¼ thick | www.usplastic.com | 45463 | 6 |
| 18. | Nine-piece Dissecting kit | VWR | 25640-002 | 2 |
| 19. | Acetone, GC-grade, 99.5% | VWR | VW0370-7 | 1 |
| 20. | Nitric acid (50%) | VWR | VW3335-1 | 2 |
| 21. | Mortar, 65 mL, case of 18 | VWR | 50420-223 | 2 |
| 22. | Mortar, 145 mL, case of 12 | VWR | 50420-245 | 1 |
| 23. | Pestle, case of 18 | VWR | 50420-427 | 2 |
| 24. | Pestle, case of 12 | VWR | 50420-449 | 1 |
| 25. | Nitrile gloves, small, box | VWR | 40101-344 | 2 |
| 26. | Nitrile gloves, medium, box | VWR | 40101-346 | 2 |
| 27. | Nitrile gloves, large | VWR | 40101-348 | 2 |
| 28. | Hydrosorb 1 12x12, 150 count | VWR | 33344-044 | 1 |
| 29. | Griffin Low form Beaker, 4L | Nalgene Labware | 1501-4000 | 2 |
| 30. | MVE Doble 28 Cryo shipper | MVE | MVE11527730 | 2 |
| 31. | MVE Doble 28 shipping container | MVE | ? | 2 |
| 32. | No.2 graphite pencil | local store | ? | 10 |
| 33. | Liqui-Nox Phosphate-Free Detergent | VWR | 21837-005 | 2 |
| 34. | Double distilled water, 30L | ? | ? | |
| 35. | Clip-n-seal, large | www.welchfluorocarbon.com | large | 1pk |
| 36. | 5 mm Teflon Layflat bags | www.welchfluorocarbon.com | P00017-1 | 1pk |
| 37. | Empty, black 35mm film canisters with lid | | | 80 |
| 38. | Cable ties, 6-8 cm in length | | | 80 |
| 39. | Cable ties, 30 cm in length | | | 4 |
| 40. | Aluminum carabiner 6-10 cm in length | | | 4 |
| 41. | Igloo Marine 54 cooler | www.igloo-store.com | | 2 |
| 42. | Collapsible fish trap 32 in. x 24 in. | www.nylonnet.com | FT-B | 3 |

Need access to Liquid nitrogen and dry ice.

## 1.1.2 Sample Nomenclature

Samples will be identified by three category designations: Site, Species, and order of collection.

All sites are designated by a capital letter. For example, the site in Barbareta, Honduras will be referred to in all collections as "A"; the site in Turneffe, Belize will be designated as "C". Species in the collection will be designated as "X", "Y", or "Z".

"X" = *Porites asteroids*
"Y" = *Strombus gigas*
"Z" = *Haemulon plumieri*

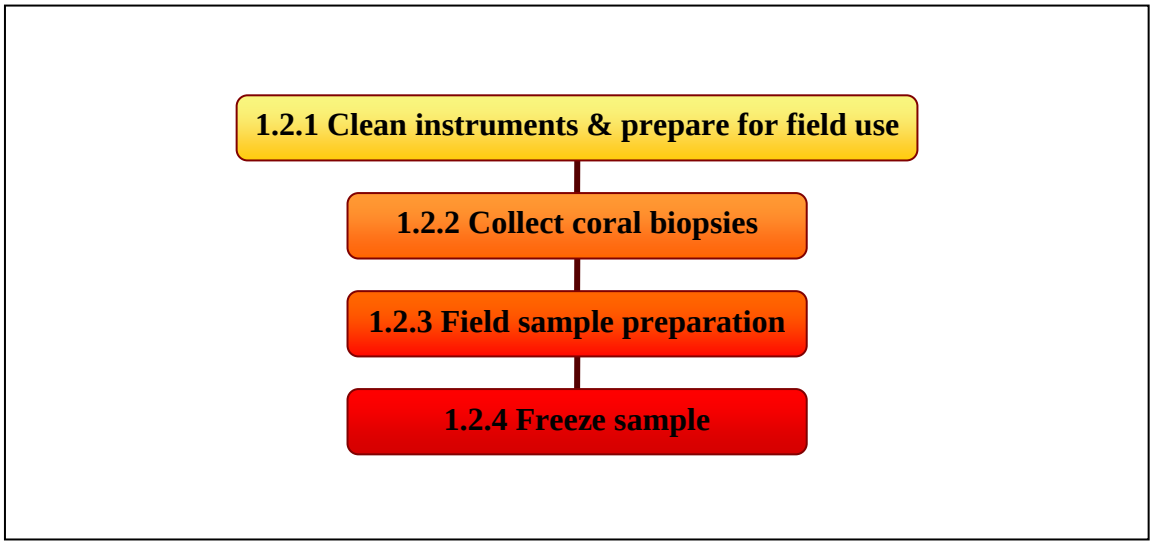
Individual samples collected from each site from any species will be designated as a number; the designation reflected by the numerical order it was collected. For example, the first coral sample collected in Barbareta will be designated as "1". The second coral sample collected in Barbareta will be designated as "2", etc… The first grunt sample collected in Barbareta will be designated as "1", etc…

Hence, all samples in shipping/archival containers, notebooks, and manifests will be identified by the designation:

[Letter (site), Letter (species), Number (sample)]

For example, the first coral sample collected in Barbareta will be designated and labeled as [AX1]. The second coral sample collected in Barbareta will be designated and labeled as [AX2], etc… The first and second Queen Conch samples collected at Tuneffe will be designated and labeled [CY1] and [CY2], respectively.

## 1.2 Sample Collection & Preparation for Coral




**1.2 Operations Chart for Coral Collection & Preparation for Contaminant Chemistry Analysis**

**Materials Needed:**

- Al Stohlman Brand Drive Punch 13/64”
- Stainless steel dissection probe
- Heavy-duty Aluminum Foil
- 5 mm Teflon Layflat bags
- 35 mm film canisters with lids
- Both sizes of cable ties
- Aluminum carabiner
- Clip-n-seal, large
- Liqui-nox detergent
- Pencil
- Acetone
- Double Distilled water
- Squirt bottle

### 1.2.1 Preparation for Cleaning

Arrange equipment and materials so they can be easily accessed once you start the cleaning process. Once you start cleaning do not touch any other equipment or surfaces with the gloves you are wearing. If you must, remove the gloves and put on a new pair of clean gloves. Any stray contamination introduced during the cleaning process will affect the subsequent analysis.

1. Fill a new squirt bottle with acetone. Identify the bottle with “acetone”.
2. Fill a new squirt bottle with the distilled water. Identify the bottle with “water”.

3. Fill a new squirt bottle with a 70% solution of acetone (ratio of 70 ml acetone to 30 ml distilled water). Identify the bottle with "70%".
4. Mix up a solution of 10% Liqui-nox detergent and place in a wash pan.
5. Place the roll of aluminum foil on a counter or table for cutting.
6. Cut a 30 cm x 30 cm section. In the top left-hand corner use a #2 pencil to inscribe an "A". The letter will let you know which side you have cleaned later.
7. Cut a piece of foil into 10 - 10 cm x 20 cm pieces. Use the pencil to inscribe an "A" in one corner of each piece.
8. Cut a piece of foil into 24 – 6 cm x 6 cm pieces. Use the pencil to mark the middle of each foil square with a sample designation number (e.g., [AX1], [AX2], [BX1], etc.) There should be 24 separate designation numbers.
9. Put on a clean pair of nitrile gloves.
10. Wash the side of the 30 cm x 30 cm piece of aluminum foil, with the "A", with acetone using the squirt bottle.
11. Wash one side of each piece of 10 cm x 10 cm aluminum foil with acetone using the squirt bottle. Set the unwashed side face-down on a clean counter. Allow the foil to dry (15 minutes).
12. Rinse the forceps with acetone. Use the forceps to pick up each 6 cm x 6 cm piece of foil for cleaning. Wash both sides of the foil square with acetone using the acetone-squirt bottle. Allow to air dry on a 10 cm x 20 cm piece of acetone-rinsed aluminum foil.
13. Allow all pieces of foil to air dry, about 15 minutes.
14. Acetone rinse four Oak Ridge 50 mL Teflon tubes/caps. Allow to air dry, then seal the tube.


Figure 1. Basic configuration design of 35 mm film canister loop for coral sampling

### 1.2.2 Clean Instruments & Prepare for Field Use

1. Wearing the nitrile gloves, wash the Drive Punches and Dissection probes in the 10% Liqui-nox detergent.
2. Remove all residual detergent on the instruments by rinsing the instruments with distilled water using the squirt bottle.
3. Rinse instruments with acetone using the squirt bottle.
4. Place instruments on acetone-cleaned aluminum foil, allow to dry completely.
5. Place one Drive Punch and one Dissection Probe onto a single 10x20 cm aluminum foil piece (on the side of the foil that was washed with acetone).
6. Wrap the instruments with the acetone-cleaned aluminum foil.
7. Place one aluminum-covered instrument set into Teflon Layflat bag.
8. Seal the Layflat bag.
9. Punch a single hole in the lid of the 35 mm canister using the point of a sharp knife, about 1 mm in diameter and about 4 mm from the edge of the lid.
10. Punch a single hole in the side of the 35 mm canister, about 1 mm in diameter, and about 5 mm below the lip of the canister.
11. Punch a single hole at the bottom of the 35 mm canister, about 2 mm in diameter, and in the center.
12. Loop the lid and canister together with the 6-8 cm long cable tie. Make sure not to constrict the cable tie loop too tightly. The lid must be able to come off, and be put back on, the canister easily.
13. Quickly rinse the inside of the canister and the inside of the lid with the 70% acetone solution, followed very quickly with washing with double distilled water. Acetone can melt plastic.
14. Allow to air dry, then cap the containers.
15. Number each canister with a number, from 1 to 6, by etching the number to the outside of the canister with a sharp knife.
16. Assemble 6 canister together by looping the 30 cm cable tie through the 6-8 cm cable tie loop (see Figure 1).
17. Gather the six sample-foil squares that pertain to a single sampling site into a Teflon Layflat bag that has been rinsed with acetone/air dried. Seal the bag.
18. Identify the four Oak Ridge 50 mL Teflon tubes/caps. Using a Dremmel with a fine point or a knife, etch the site and species designation onto the side of the tube. Each tube should have a different designation code.
19. Finally, place a carabiner onto the 30 cm cable-tie loop (see Figure 1).

### 1.2.2. Collection of Coral Biopsies

Collection of samples is assumed to be conducted by SCUBA diving off a boat. There should be three people for the collection, the two collection divers and the ‘boat person’.

1. Once collection divers have donned their dive gear, divers should put on nitrile gloves. The 'boat person' will latch the 35 mm film canister to the wing diver's buoyancy control device using the carabiner.
2. Remove a single set of drive punch/dissecting probe from its protective covering; the drive punch and the hammer are taken by the lead diver.
3. The wing diver will bring with them the dissecting probe.
4. Once at the target site, the lead diver will place the drive punch on the apex of the coral colony. The apex of the coral is the area of the coral that is perpendicular to the surface of the water, and should receive the greatest amount of incident light (Figure 2). Do not collect the biopsy from the coral edge, as you may collect non-coral tissue, thereby introducing significant artifact into the study (Figure 3).
5. With several taps with the hammer, the drive punch should be driven into the colony about 1 cm. The diver must go deep enough into the coral colony so that the biopsy contains all visible tissue (Figure 4). For most boulder corals, the tissue can extend into the skeleton from 1mm to as much as 10 mm. If boring sponge is present, try not to collect the sponge with your biopsy – resample from the same coral colony, or sample from a different coral colony. The coral biopsy will remain in the drive punch.
6. The lead diver hands the drive punch that contains the biopsy to the wing diver. The wing diver, using the dissecting probe, pushes out the coral biopsy into the open 35 mm film canister. Once the biopsy is in the canister, seal the canister with the lid.
7. Wing diver will then return the drive punch to the lead diver.
8. Ideally, every sample should have a new drive punch/probe, though in most cases, this many not be financially possible. If this constraint is present, repeat sampling procedure with the next coral colony using the same coring device for all colonies at a given site.

Figure 2. Place drive punch at the apex or very near the absolute apex of the coral colony. The biopsy lesion should be completely surrounded by healthy coral tissue. Unlike this picture, diver shall wear nitrile gloves.

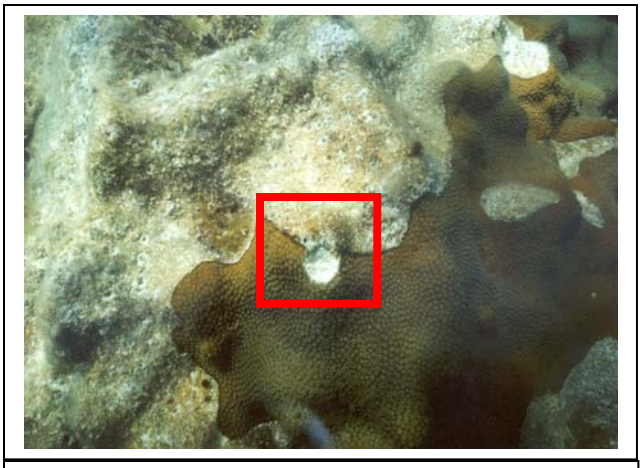


Figure 3. The biopsy SHOULD NOT be taken from tissue that is near the edge of the coral colony

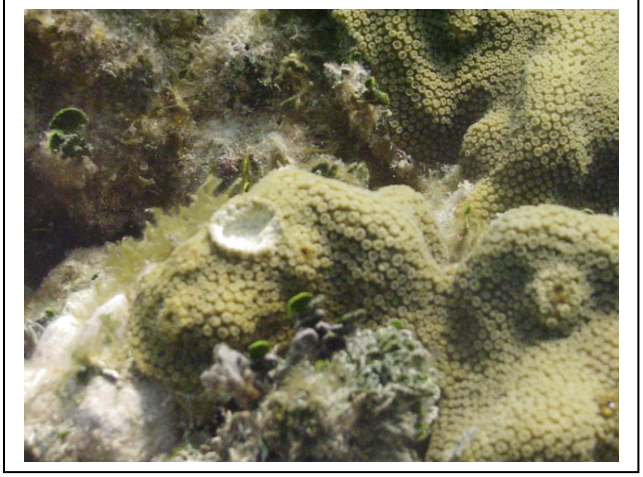


Figure 4. The divot created by the biopsy should tell you whether or not your biopsy went deep enough into the coral skeleton. You should see that the bowl of the divet extends below the tissue layer by about 2-3 mm.

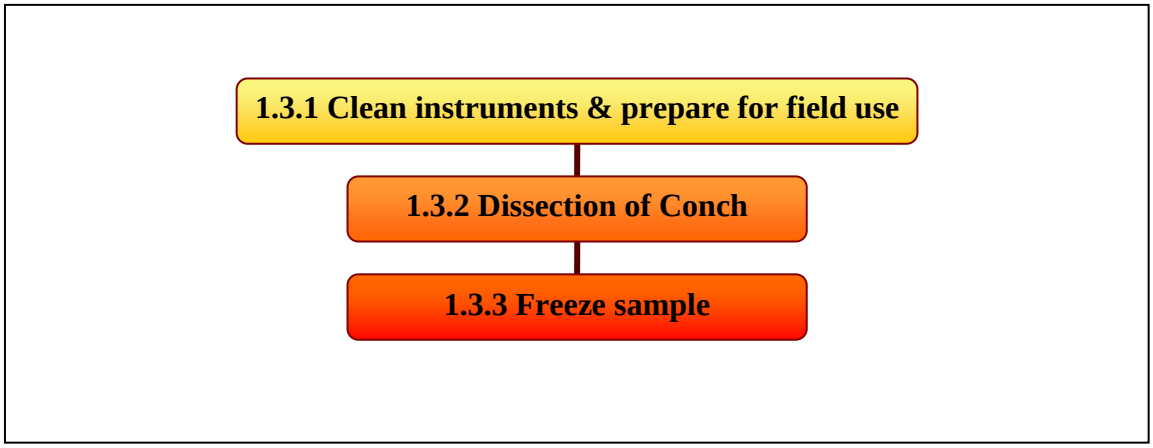
### 1.2.3. Field Sample Preparation

1. During the diver interval, the boat person should make ready the 6 cm x 6 cm foil squares, a clean surface area to work on (a sheet of acetone-washed aluminum foil laid across the top of the igloo marine cooler). Boat person should be wearing clean nitrile gloves.
2. At the surface, the wing diver hands the film canister loop to the boat person.
3. Opening a single film canister, the boat person removes the coral biopsy from the canister using acetone-rinsed forceps. Place biopsy on one of the 6 cm x 6 cm foil squares, place sample on top of the penciled number. Fold the foil square over the biopsy and crease with sharp edges and a rolled/pinched seal.
4. Place foiled biopsy into the 50 mL Oak Ridge Teflon centrifuge tube; the tube that is designated for this sampling site.
5. All six samples should fit into the single centrifuge tube.
6. Place the cap back onto the centrifuge tube, but only give the lid ¾ of a full rotation. An air-tight seal on the centrifuge tube could result in an exploding tube when the tube is removed from the cryo-vapor shipper.

### 1.2.4. Freeze Sample

1. Place the tube in the cryo-vapor shipper or in the Igloo Marine Cooler that contains dry ice.

## 1.3. Sample Preparation & Collection for Queen Conch




## Materials

- Stainless steel hammer with a sparred end or stainless steel chisel
- Scalpel
- forceps
- Heavy-duty Aluminum Foil
- Oak Ridge Centrifuge tube, 30 mL
- 5 mm Teflon Layflat bags
- Clip-n-seal, large
- Liqui-nox detergent
- Acetone
- Double Distilled water

If you have a stainless steel hammer with sparred end, you do not need the stainless steal chisel, and vice versa.

### 1.3.1 Clean instruments & prepare for field use

1. Wear nitrile gloves during the entire procedure.
2. Wash one side of a 30 cm x 30 cm piece of aluminum foil with acetone using the squirt bottle.
3. Allow sheet to airdry on the counter (15 minutes).
4. Wash the hammer/chisel, forceps and scalpel in 10% Liqui-nox detergent (dilute with double distilled water).
5. Remove all residual detergent on the instruments by rinsing the instruments with double distilled water using the squirt bottle.
6. Rinse instruments with acetone using the squirt bottle.
7. Place instruments on acetone-cleaned aluminum foil, allow to dry completely.
8. Cut aluminum foil into 30 cm x 20 cm pieces.

9. Wash one side of each piece of aluminum foil with acetone using the squirt bottle. Set the unwashed side face-down on a clean counter. Allow the foil to dry (15 minutes).
10. Place one hammer/chisel and one scalpel onto a single 30x20 cm aluminum foil piece (on the side of the foil that was washed with acetone).
11. Wrap the instruments with the acetone-cleaned aluminum foil.3
12. 
13. Place aluminum-covered instrument set into Teflon Layflat bag.
14. Seal the Layflat bag.
15. Acetone rinse 32 Oak Ridge 30 mL Teflon tubes/caps. Allow to air dry, then seal the tube.
16. Using a dremmel with a fine point or a knife, etch the site, species, and sample number designation onto the side of the tube. Each tube should have a different designation code.

### 1.3.2 Dissection of Conch

1. Once the conch has been collected, it can placed in the cooler on dry ice, or it can be dissected in the field.
2. Whether dissection occurs in the field or back in the lab, acetone clean one side of a 30 cm x 30cm piece of aluminum foil, and place the uncleaned side down on a clean benchtop or Teflon cutting board.
3. Wear nitrile gloves.
4. Position the conch shell on a clean surface and puncture or "crack" the shell with the stainless steel hammer or chisel at the location indicated in **Figure 5**.


**Figure 5**. Puncture or "crack" the shell at the location indicated in the figure. Use an acetone-cleaned stainless steel hammer with a spar or a stainless steel chisel.

5. With the scalpel, cut the retractor muscle off of the shell (**Figure 6**).
6. Pull the conch out of the shell.
7. Cut a 7 cm long x 1 cm in diameter swath of mantle tissue using the acetone-cleaned scalpel and forceps (**Figure 7**).
8. Place the mantel tissue into the labeled Oak Ridge Telfon 30 mL centrifuge tube that is appropriately labeled.

9. Be consistent with the area from which you dissect the tissue from one sample to another.


**Figure 6**. Using the acetone-cleaned scalpel, cut the retractor muscle from the shell so that the conch can be removed easily from the shell.

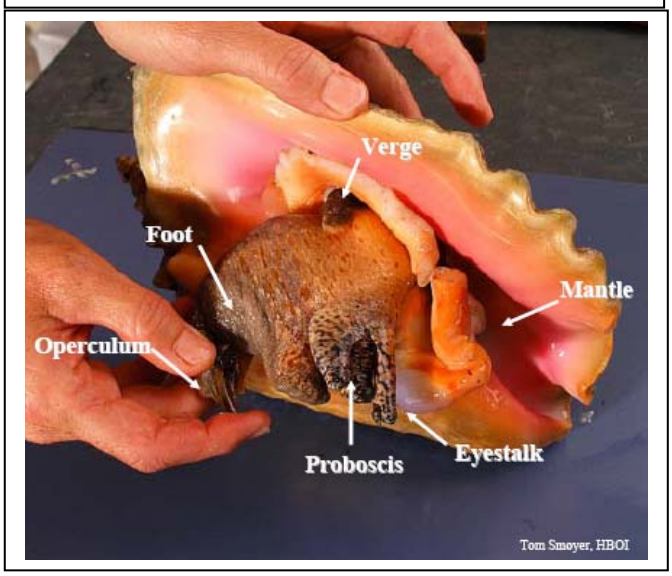
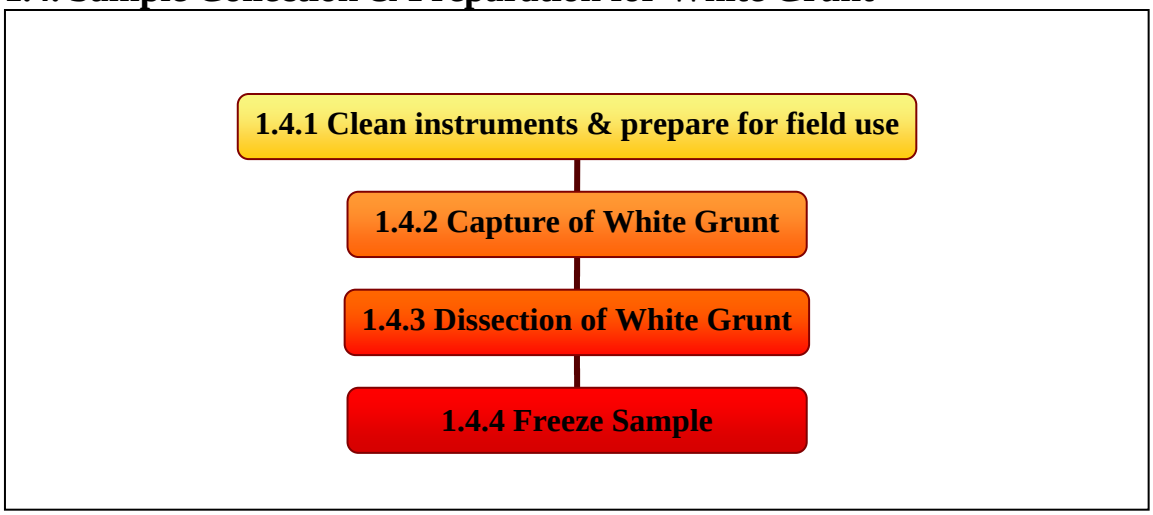



**Figure 7**. Cut a 7 cm long and about a 1-2 cm wide swath of mantle tissue using the acetone-cleaned scalpel and forceps.

### 1.3.3 Freeze sample

1. Place the tube in the cryo-vapor shipper or in the Igloo Marine Cooler that contains dry ice.

## 1.4. Sample Collection & Preparation for White Grunt




## Materials

- Fish cages
- Scalpel
- Forceps
- Surgical scissors
- Heavy-duty Aluminum Foil
- Oak Ridge Centrifuge tube, 10 mL
- Teflon 12x12 cutting boards
- 5 mm Teflon Layflat bags
- Clip-n-seal, large
- Liqui-nox detergent
- Acetone
- Double Distilled water

### 1.4.1 Clean instruments & prepare for field use

1. Wear nitrile gloves during the entire procedure.
2. Wash one side of a 30 cm x 30 cm piece of aluminum foil with acetone using the squirt bottle.
3. Allow sheet to airdry on the counter (15 minutes).
4. Wash the surgical scissors, forceps, and scalpel in 10% Liqui-nox detergent (dilute with double distilled water).
5. Remove all residual detergent on the instruments by rinsing the instruments with double distilled water using the squirt bottle.
6. Rinse instruments with acetone using the squirt bottle.
7. Place instruments on acetone-cleaned aluminum foil, allow to dry completely.
8. Cut aluminum foil into 30 cm x 20 cm pieces.
9. Wash one side of each piece of aluminum foil with acetone using the squirt bottle. Set the unwashed side face-down on a clean counter. Allow the foil to dry (15 minutes).

10. Place one surgical scissors, forceps, and scalpel onto a single 20x20 cm aluminum foil piece (on the side of the foil that was washed with acetone).
11. Wrap the instruments with the acetone-cleaned aluminum foil.
12. Place aluminum-covered instrument set into Teflon Layflat bag.
13. Seal the Layflat bag.
14. Acetone rinse 32 Oak Ridge 10 mL Teflon tubes/caps. Allow to air dry, then seal the tube.
15. Using a dremmel with a fine point or a knife, etch the site, species, and sample number designation onto the side of the tube. Each tube should have a different designation code.

### 1.4.2 Capture of White Grunt

1. Purchase bait from store.
2. Using an acetone-cleaned instrument, place approximately 5-10 grams of bait into a Oak Ridge Telfon 10 mL tube, and etch onto the side of the tube, "Fish bait." Freeze bait sample in -20C freezer or in the cryo-dry shipper.
3. When at the site location, place bait in trap.
4. Have a diver set the trap at a location where a lot of white grunt are evident.
5. Allow trap to set for at least 30 minutes.
6. Seal entrance to trap, bring trap to surface.
7. Collect only White Grunts that are 25 cm and longer.
8. Wrap grunt in 36 x 36 cm aluminum foil square that has been acetoned-washed and labeled. Place Grunt directly over penciled label. Wrap Grunt in the aluminum foil square, making sure of creased edges and rolled-pinched seal.
9. Freeze same in Igloo Marine cooler with dry ice in it.
10. The fish will most likely exhibit intensive physical activity when you try to wrap the fish in foil, making it difficult and frustrating to carry out the procedure. There are a number of ways to reduce this effort. It is important to keep in mind that whatever method of reducing the physical exertion of the fish should be legally acceptable and institutionally approved. Placing the fish in a acetone cleaned tank with site water and a fish anesthetic may be the easiest and most socially acceptable method.

### 1.4.3 Dissection of White Grunt

1. Place acetone-cleaned 12x24 teflon cutting board on a level and clean surface.
2. Partially thaw fish at room temperature (e.g., room temperature = 25°C-29°C).
3. Wear nitrile gloves.
4. Remove fish from aluminum foil covering and place on 12x24 cm Teflon cutting board.
5. Head of fish should be facing to your right, the tail facing to your left.
6. Make an incision with the scalpel that run from the dorsal edge to the bottom of the belly; the incision should be made in front of the pectoral fin (**Figure 8**).

7. Make a second incision that begins where the first incision ended at the fish's belly, and run along the belly to the vent (**Figure 8**; **Figure 9**). If it is easier for you, use the surgical scissors to make the cut.
8. Make a third incision from the vent across the side of the fish, ending the incision at the site of the beginning of the first incision (**Figure 8; Figure 10**).

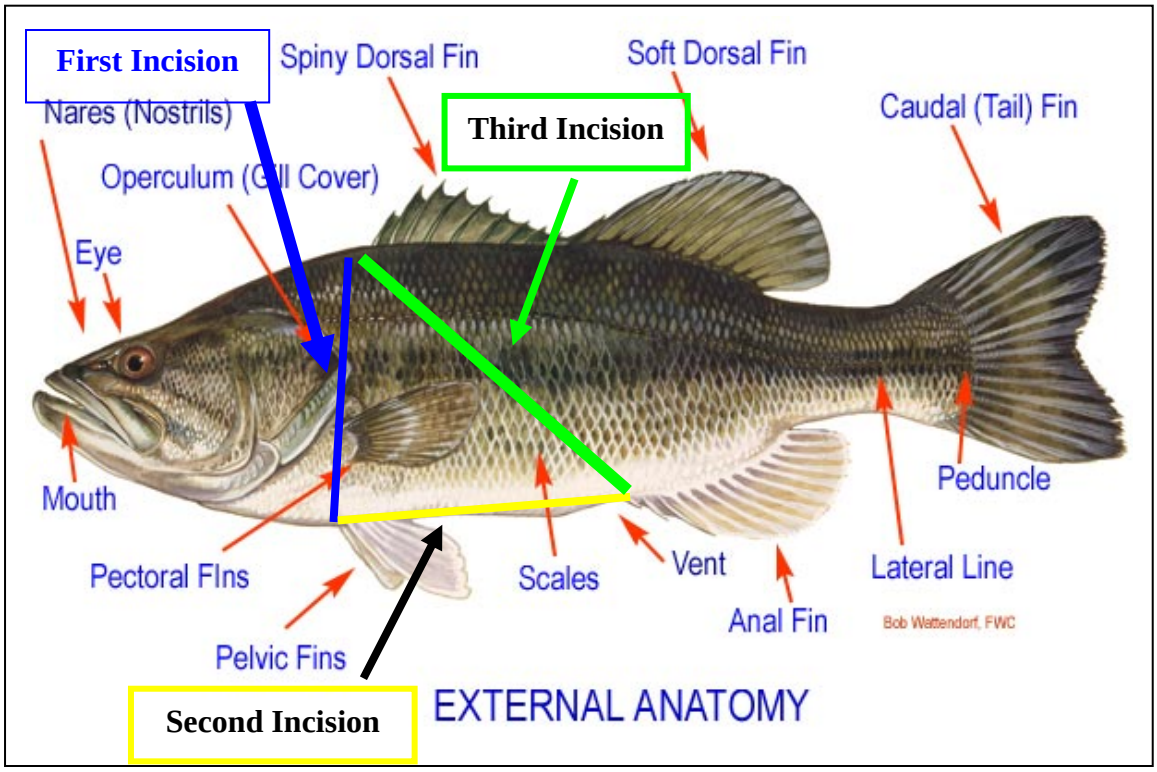



**Figure 8.** Make an incision along the blue line. The cut should only be deep enough to penetrate the muscle and break the gastrovascular fascia. DO NOT CUT into the organs. The yellow line is the second incision; again, penetrate the muscle and fascia, but do not cut into the organs. For the second incision, you may use your surgical scissors. The green line is the third incision; again, penetrate the muscle and fascia, but do not cut into the organs.

**Figure 9.** Second incision along the belly

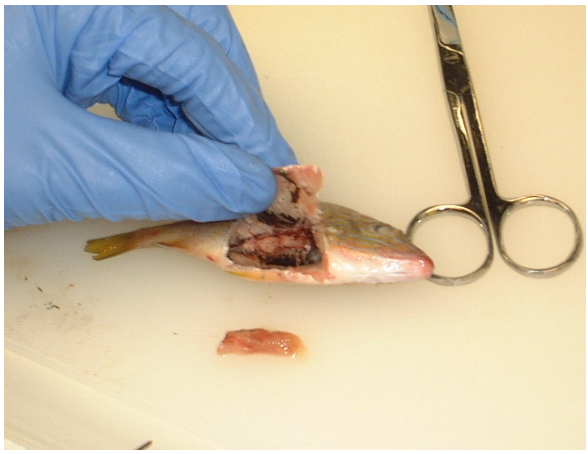


**Figure 10.** Third incision along the side

9. Identify the liver. It should be dark-red in color, and near the gills and immediately below the stomach (**Figure 11**).
10. Remove the liver with forceps and scalpel.
11. Place liver in acetone-cleaner Oak Ridge Telfon 10 mL centrifuge tube. Cap the tube with the lid, making sure that the lid is NOT on tight.

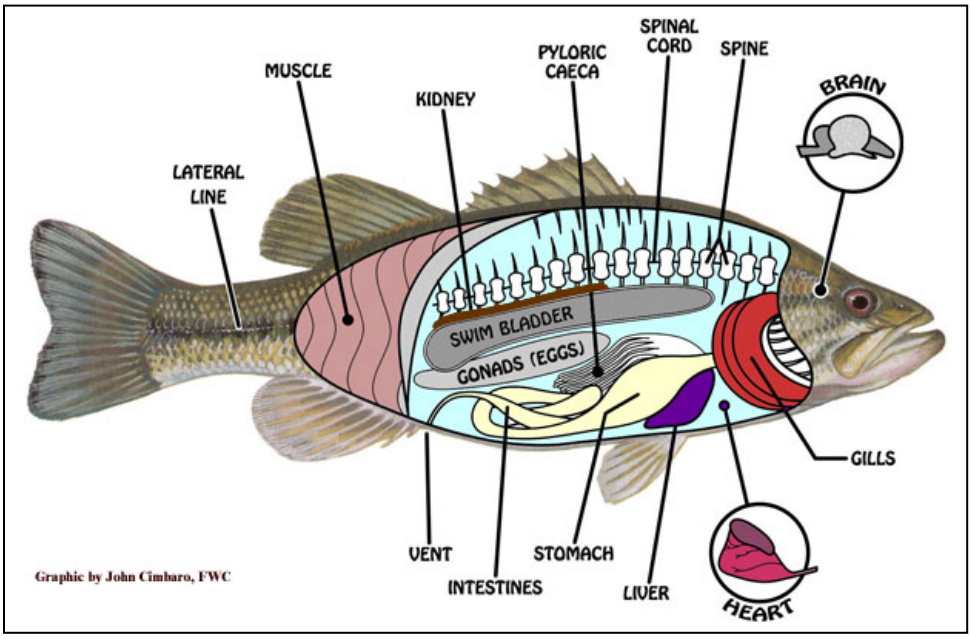



**Figure 11**. Liver is dark-red in color, and between the stomach and the gills

### 1.4.4 Freeze Sample

1. Place sample in cryo-dry shipper or in a -20C freezer. Samples can only remain at -20C for 28 days.

## 1.5. Sample Collection of Sediment for Organic Contaminant Analysis

### Materials

Sediment Jars, 500 mL

### 1.5.1. Collection Procedures

The particle composition and size of a sediment has a direct bearing on the inferences that can be made from the contaminant chemistry data resulting from a sample site. The types of sediment at the sampling sites are not known to the author, and a search of the literature to the best of the author's ability resulted in no relevant scientific publications concerning sediment characterization of the sampling sites as it relates to tenets of the Particulate Sampling Theory (http://www.epa.gov/swerust1/cat/mason.pdf).

With the inherent limitations placed on this project by funding and expertise resources, the author suggests that, in order to bestow a measure of consistency in the comparison of contaminant loads of sediments between and among sites, particulate size within a sediment sample should be, in average, small then two millimeters.

The author also suggests that the divers make note in the field book a description of the texture, color and scent of the matrix of each sediment sample.

1. At each sampling site, place six jars into mesh bag. Jars should have lid on them. If you do not dive deeper than 15 meters, the pressure on the jar should not break the jar. Do loosen the lid on the jar so that it can be opened easily underwater.
2. Diver should wear nitrile gloves.
3. At depth, open jar above sediment, and scoop the sediment into the jar using the lip of the jar. Only collect the top 1-2 centimeters of sediment.
4. Try to ensure that the jar is completely filled with sediment, and contains little residual seawater.
5. Cap jar with lid.
6. Once on deck, label the jar with a permenant marker or with laboratory tape, and chill the sample. Place jar in an Igloo cooler with normal ice – not DRY ICE.
7. If possible, open the jar and decant any excess seawater.
8. Once back in the lab, place the jar in a -20°C freezer.

If possible, grain size distribution for each sediment sample should be determined so that contaminants analysis results can be normalized against this grain size distribution. Grain size should be determined either by sieve fractionation or by gravimetric pipetting. The major fractions of particle distribution (based on the Wentworth scale) include the following classes:

- cobble (-6 phi and above)
- gravel (-2 phi to -5 phi)
- sand (+4 phi to -1 phi)
- combination of both silt and clay (+5 phi and below)

## 2.1. Option 1 - Contaminant Sample Analysis

Option 1 assumes that none of the sample will be archived or sent to a secondary analysis lab.

Samples from all three species will be in their Teflon, capped, centrifuge tubes frozen in the cryo-vapor shipper.

1. Create a manifest of all contents in the cryo-vapor shipper.
2. Obtain the necessary export CITES permits.
3. Obtain the necessary import CITES permits.
4. DO NOT USE **DHL** as a courier service. Use air freight with an airline, FEDEX, or an air-freight service.
5. The day before shipping, make sure the cryo-vapor shipper is charged.
6. Notify the receiver five days in advance, then again at 48 hours in advance, before the day the cryo-shipper is being sent to the receiver.
7. It is best that the receiver meets personally with the U.S Fish and Wildlife and the U.S. Customs inspection agents during the inspection of the cryo-shipper to expedite the processing and ensure that the cryo-shipper is delivered to the receiver in a timely fashion.

## 2.2 Option 2 - Long-term management of contaminant sample analysis

Option 2 is a long-term management plan for samples collected as part of the Meso-American reef system. Option 2 is based on U.S. EPA's split-sample analysis interlaboratory program. Samples are collected and prepared (as described in Section 1) and shipped to a central processing and storage facility. Samples are ground frozen to a fine powder, and aliquoted into four separate tubes. The purposes of each tube are as follows:

- Tube 1 is sent to the primary contaminant-chemistry analysis facility.
- Tube 2 is archived for future reference.
- Tube 3 can be sent to the secondary contaminant-chemistry analysis facility if there is a dispute, or for analysis of target analytes that are not included in the primary contaminant-chemistry analysis.
- Tube 4 is for biological marker (biomarker) analysis
- Each tube will be given a new identification code that will ensure that all of the analysis facilities will be 'blind' to the true identification of the sample.

This Central Processing and Storage Facility is required to have the following items

- -80C of liquid nitrogen freezer storage
- Laminar flow clean hood
- Back-up power supply for cryo/freezer storage
- Secure entry
- System tracking of samples/identification
- Sample preparation equipment and supplies

**Procedures for Option 2**

**2.2.1 Clean Instruments and prepare for use**

1. Ship samples to Central Processing and Storage Facility as in Option 1.
2. Samples processed by ID input into data management system and stored either in a -80°C freezer or in a liquid nitrogen cryo-storage container.
3. Mortar and pestles are washed in Liqui-nox detergent, rinsed in distilled water.
4. Mortar and pestles are set in a solution of 50% nitric acid for 2 hours.
5. Mortar and pestles are liberally rinsed in distilled water.
6. Mortar and pestles are rinsed with acetone.
7. Mortar and pestles are air-dried for five minutes, then wrapped in acetone-cleaned aluminum foil for storage.
8. Stainless steel spatulas are washed with Liqui-nox detergent, rinsed with distilled water, then washed with acetone and air-dried. Wrap spatulas in aluminum foil for storage.

**2.2.2. Sample preparation**

1. Wear nitrile gloves.
2. Unwrap mortars, pestles, forceps, and spatulas from their aluminum foil covering in the clean hood.
3. In the 4 mL-Teflon sample tubes with screw caps, appropriately label the side of each tube with the Sample ID, and the storage tube Number (1-4).
4. Place the four 4 mL Teflon sample tubes into the tube racks, and place the racks in the ceramic 'cool' box with dry ice in the box. This is so that once you put the ground, frozen sample into each of the tubes, the samples do not thaw.
5. Place the 'cool' box with tubes into the clean hood. It is assumed that the cool box and the tube racks have been cleaned with Liqui-nox detergent, rinsed in distilled water, and acetone-washed.
6. Fill cryo-dewar with liquid nitrogen, and place dewar into clean hood.
7. Remove the samples from the cryo-storage container and place in a second 'cool' box that is filled with dry ice.
8. Set the second 'cool' box with the sample in it on a table next to the clean hood.
9. Pour liquid nitrogen into a mortar that has the pestle in it. Fill the mortar to the rim, and allow the mortar to boil off (**Figure 12**).
10. Again, pour liquid nitrogen into a mortar that has the pestle in it. Fill the mortar to the rim, and allow the mortar to boil off.
11. Rest the pestle on the lip of the mortar, and for the third time, pour liquid nitrogen into a mortar (**Figure 13**).
12. As the liquid nitrogen is boiling in the mortar, remove the sample from its 3114 Oak Ridge Teflon Centrigue tube with a forcep, and place the entire sample into the mortar that has liquid nitrogen in it.
13. With the pestle, fracture the sample into smaller pieces before the liquid nitrogen completely boils off.
14. With 1-4 mL of liquid nitrogen remaining in the mortar, quickly grind the sample into the mortar applying significant pressure. Continue to do this until about 5-10 seconds after the liquid nitrogen completely boils off from the mortar.

15. Carefully add liquid nitrogen into the mortar, filling it to 1/3 to ¼ full.
16. Carefully grind the samples into a powder that is of a granulated consistency between ground sugar and finely milled flower (**Figure 14**).
17. With an acetone-clean, liquid-nitrogen cooled spatula, pour contents of the mortar equally into the four sample tubes that are in the 'cool'box with dry ice in it.
18. Cap the samples, but do not make the seal air-tight, as nitrogen gas that is escaping from the sample can build up pressure within the tube and burst the tube.
19. Place sample in -80C freezer or cryo-storage facility.

**Figure 12**. Pour liquid nitrogen into the mortar/pestle configuration and fill to the brim of the mortar

**Figure 13.** For the third filling of quid nitrogen into the mortar, remove the pestle and rest the pestle on the lip of the mortar.

**Figure 15**. Grind samples into a fine powder, making sure that the sample never thaws. Be careful when pouring liquid nitrogen into the mortar with granulized sample, as the liquid will sometime splash the sample out of the mortar and onto the bench.

# 3. Contaminant Chemistry Protocol

Contaminant chemistry analysis for both tissue and samples is assumed to be conducted using gas chromatograph (GC) using an electron-capture detector. Extraction procedures for both tissue and sediment can be done using standardized liquid-solid extraction methods, such as the Soxhlet Extraction method (EPA Method 3540) or an Accelerated Solvent Extraction (ASE) method (EPA Method 3545). Other extraction methods can also be used, such as Microwave Accelerated Extraction Method (EPA Method 3546) and Super-Critical Fluid Extraction Method. The Soxhlet and ASE methods seem to predominate in labs within the Meso-American Reef system and it is suggested that participating laboratories use the Soxhlet and ASE methods.

Samples will be normalized against wet weight and against lipid weight so that the data can be compared to the data in other studies.

## 3.1. Normalization

### 3.1.1. Gravimetric determination of lipid content of tissue sample

***As noted by the below quote from the EPA document (Guidance for Assessing Chemical Contaminant Data for Use In Fish Advisories. Volume 1: Fish Sampling and Analysis - Third Edition), standardization of an extraction solvent is paramount. Based on current accepted methods in the literature, the broad array of difference extraction methods, and the availability of obtaining pesticide-grade solvents in the Meso-American Region, North America, and South America, I recommend that all laboratories that participate in this study use dichloromethane mix as the initial extraction solvent (EPA Method 8290 and 1613).***

*It is recommended that a gravimetic method be used for lipid analysis. This method is easy to perform and is commonly used by numerous laboratories, employing various solvent systems such as chloroform/methanol (Bligh and Dyer, 1959), petroleum ether (California Department of Fish and Game, 1990; U.S. FDA, 1990), and dichloromethane (NOAA, 1993a; Schmidt et al., 1985). The results of lipid analyses may vary significantly (i.e., by factors of 2 or 3), however, depending on the solvent system used for lipid extraction (Randall et al., 1991; D. Swackhamer, University of Minesota, personal communication, 1993; D. Murphy, Maryland Department of the Environment, Water Quality Toxics Division, personal communication, 1993). Therefore, to ensure consistency of reported results among fish contaminant monitoring programs, it is recommended that dichloromethane be used as the extraction solvent in all lipid analyses.*

*In addition to the effect of solvent systems on lipid analysis, other factors can also increase the inter- and intralaboratory variation of results if not adequately controlled (Randall et al., 1991). For example, high temperatures have been found to result in decomposition of lipid material and, therefore, should be avoided during extraction. Underestimation of total lipids can also result from denaturing of lipids by solvent contaminants, lipid decomposition from exposure to oxygen or*

*light, and lipid degradation from changes in pH during cleanup. Overestimation of total lipids may occur if a solvent such as alcohol is used, which results in substantial coextraction of nonlipid material. It is essential that these potential sources of error be considered when conducting and evaluating results of lipid analyses.*

*NOAA (National Oceanic and Atmospheric Administration). 1993c. Sampling and Analytical Methods of the National Status and Trends Program, National Benthic Surveillance and Mussel Watch Projects 1984-1992. Volume IV. Comprehensive Descriptions of Trace Organic Analytical Methods. NOAA Technical Memorandum NOS ORCA 71. Coastal Monitoring and Bioeffects Assessment Division, Office of Ocean Resources Conservation and Assessment, National Ocean Service, Silver Spring, MD. July.*

*Randall, R.C., H. Lee II, R.J. Ozretich, J.L. Lake, and R.J. Pruell. 1991.Evaluation of selected lipid methods for normalizing pollutant bioaccumulation. Environ. Toxicol. Chem. 10:1431-1436.*

Due to the difference in laboratory equipment and extraction methods, a single standardized protocol may be difficult to implement. Instead, I provide an example using a single method (one that is conducted in my laboratory) that demonstrates the principle of obtaining the parameter of wet weight and lipid weight.

- Measure out five grams of fish on a pre-tared aluminum weight dish or in a pre-tared 250 mL pre-cleaned glass beaker.
- Add five grams of ASE prep material (dionex p/n 062819) to the 250 mL pre-cleaned glass beaker with the fish tissue in it.
- Place mix in microwave for 2.5 minutes (depends on moisture content of sample).
- Transfer dried sample to ASE extraction cell using acetone-cleaned stainless steel spatula.
- Conduct ASE extraction (dichloromethane, pressure at 1500 psi, 105 degrees Celsius).
- After ASE processing, transfer extract to 40mL pre-cleaned glass graduated vial
- Dry extract to 10mL, measure precisely using.
- Transfer 0.5 mL (10%) to pre-cleaned, pre-tared aluminum weighing dish.
- Dry 0.5 mL to dryness and weigh dish for 10% weight of lipids from this five gram sample.

### 3.1.2. Dry weight determination of tissue sample

The purpose of this procedure is to be able to normalize the concentration of contaminants against a parameter that has consistency from one sample to another.

All instruments and glassware should be cleaned with a phosphate-free micro-detergent, and acetone rinsed. Samples will be thawed, and using a cleaned spatula, one to three grams of thawed material will be placed in a clean 10-25 mL beaker that has been pre-

tared, and pre-dried (in the drying oven at the same temperature the samples will be dried at). The 'wet-weight' of the beaker is weighed, the amount recorded.

Samples are then placed in a drying oven and incubated to dryness. Drying oven temperature should be between 100°C and 115°C. The temperature should be consistent for all incubation of samples. Samples in beakers are weighed again, the amount recorded.

Ask for certification in the accuracy of all balances and thermometers used in this procedure. Accuracy of balances and thermometer should be checked before and after processing of the samples. Also ask for guarantee that the lab cleans all instruments and glassware appropriately so as prevent the occurrence of cross-contamination of samples.

Equation for calculating the percent dry weight of a sediment sample.

$$\text{Dry Wt.\%} = \frac{(\text{Beaker Wt.} + \text{Dry Wt.}_{\text{sample}}) - (\text{Beaker Wt.})}{(\text{Beaker Wt.} + \text{Wet Wt.}_{\text{sample}}) - (\text{Beaker Wt.})} \times 100$$

### 3.1.3. Dry weight determination of sediments

The purpose of this procedure is to be able to normalize the concentration of contaminants against a parameter that has consistency from one sample to another.

All instruments and glassware should be cleaned with a phosphate-free micro-detergent, and acetone rinsed. Samples will be thawed, and about one to two grams of tissue be homogenized in a homogenizer. Once homogenized, the homogenate is transferred to a clean 10-25 mL beaker that has been pre-tared, and pre-dried (in the drying oven at the same temperature the samples will be dried at). The 'wet-weight' of the beaker is weighed, the amount recorded.

Samples are then placed in a drying oven and incubated to dryness. Incubation should be between 24-36 hours. Drying oven temperature should be between 100°C and 115°C. The temperature should be consistent for all incubation of samples. Once samples are dried, they should be allowed to cool to room temperature. This should take less than one hour. Samples in beakers are weighed again, the amount recorded.

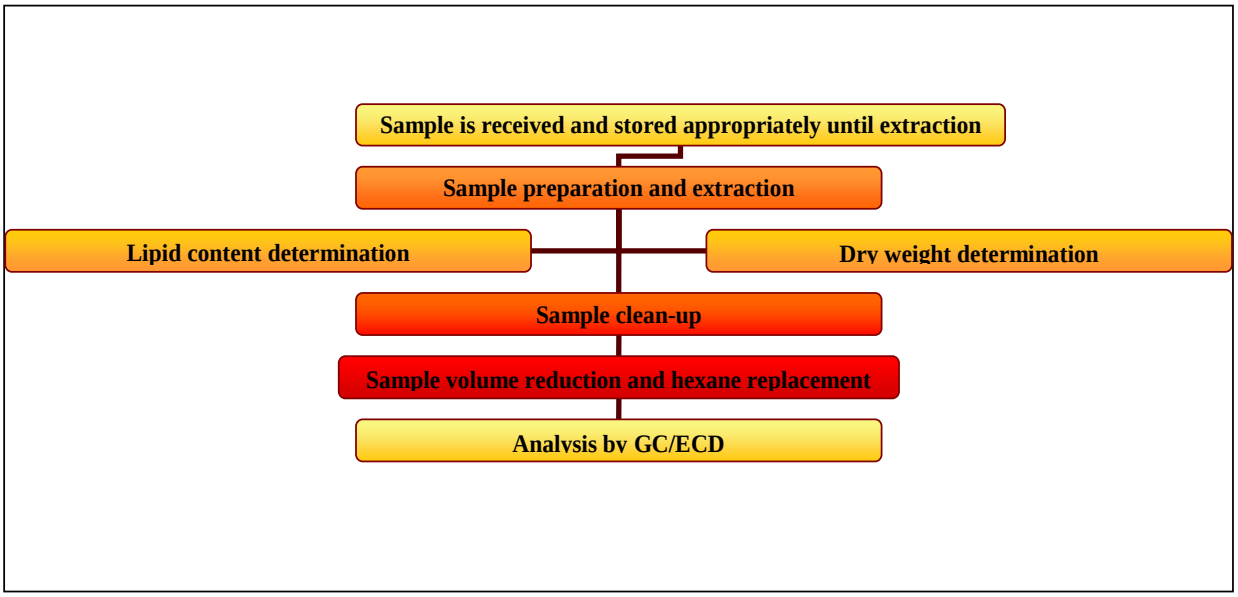
Ask for certification in the accuracy of all balances and thermometers used in this procedure. Accuracy of balances and thermometer should be checked before and after processing of the samples. Also ask for guarantee that the lab cleans all instruments and glassware appropriately so as prevent the occurrence of cross-contamination of samples.

Equation for calculating the percent dry weight of a sediment sample.

$$\text{Dry Wt.\%} = \frac{(\text{Beaker Wt.} + \text{Dry Wt.}_{\text{sample}}) - (\text{Beaker Wt.})}{(\text{Beaker Wt.} + \text{Wet Wt.}_{\text{sample}}) - (\text{Beaker Wt.})} \times 100$$

### 3.2. Example Protocol of Method

Because of the diversity of instruments and acceptable methods that can be used in the Analytical Laboratory for gas chromatography/electron capture detection, the document provides a single protocol for the extraction and detection of target analytes using a GC/ECD instrument. It can be used as a template for examining key attributes that any analytical laboratory's methodology should possess. The extraction method and the instruments used in this example *should not* be taken as that every analytical laboratory must use this method and this specific instrument. Coral will used as the sample example. The flow of the process is diagrammed below.




#### 3.2.1 Sample Preparation and Extraction

1. Corals are ground in liquid nitrogen in a mortar and pestle as described in Section 2.2.2. of this document and it is assumed that this process is being done by a competent technician in a clean lab with clean instruments.
2. About one gram of frozen powder is transferred to a clean container using a frozen spatula for determination of dry-weight.
3. Frozen sample are then dried. This step can be done using a variety of methods, but most laboratories dry samples using Hydromatrix, a commercial chemical desscicant.
4. The dried sample is then loaded into a Dionex ASE (accelerated solvent extraction) cell and using dichloromethane as the extractant solvent. All samples, blanks, matrix spikes, and reference samples are prepared in the same fashion.
5. The ASE cells are closed and loaded onto the Dionex ASE extraction instrument.
6. Extracts are collected into extraction vials, and then evaporated to a volume of about 10 milliliters using a heating block or hot-water bath.

7. An aliquot of the sample is removed (usually 100 uL to 500 uL) to determine lipid content of the sample. The method for determining lipid concentration can be found in Section 3.1.1. of this document.
8. This extract is 'cleaned' of contaminating debris and lipid. Addition of samples with high lipid content onto the GC can create significant artifact. 'Clean-up' of samples can be done using a variety of methods, and even a combination of methods. For this sample example, clean-up is conducted using alumina/silica gel chromatography columns from a commercial source that comes pre-packed and pre-equilibrated in dichloromethane. Columns are washed with a 1:1 solution of hexane:dichloromethane. The elutant is collected, and its volume reduced via evaporation. The final sample is diluted to 0.5 milliliters with hexane.
9. The sample is then transferred to a one milliliter vial and stored at -80°C until analysis.

### 3.2.2. Sample Analysis

Samples are loaded unto an autosampler that is attached to the gas chromatograph. The autosampler will inject 5 microliters of a sample into the gas chromatograph. Besides the coral samples, the autosampler will also inject method blanks, calibrant standards, matrix standards, internal standard, and the surrogate spiked standard in a single run. A run is defined as a set of samples and standards that are loaded onto the instrument over a discrete amount of time (usually 36 hours).

## 3.3. Standards

There are four different standards that any analytical laboratory must have in their methods. These four standards are necessary to provide a reasonable level of confidence in the data generated for the samples.

### 3.3.1. Internal Standard

This standard is added to all samples and quality control samples and is used to provide a reference for the calculation of analyte concentrations. Most often, the internal standard is a 1 nanogram/microliter concentration of tetrachloro-m-xylene. This compound can be obtain from a number of commercial sources and is certified/designated for use as an internal standard in contaminant chemistry analysis.

### 3.3.2. Surrogate Spiking Standard

This standard is used to determine the efficiency of analyte recovery of the method used in the analytical laboratory. The surrogate spiked standard *cannot* be a target analyte (e.g., PCB 105), and can be compounds such as 4,4,'-dibromooctoflurobiphenyl, tetrachloro-m-xylene, and 2,2',4,5',6 pentachlorobiphenyl. This compound can be obtain from a number of commercial sources and is certified/designated for use as a standard in contaminant chemistry analysis.

### 3.3.3. Calibration Standards (aka working standards)

The purpose of Calibration Standards is to calibrate the instrument and to determine the range of linearity for the instrument. These standards are purified/known concentration of the target analytes. They are obtained from a commercial source and are certified/designated for use as calibrant standards. The calibrants are actually a series of dilutions of a mastermix of target analytes are loaded directly into the GC-ECD instrument. Calibrants for each target analyte may range from 1 to 500 picograms/microliter. For organochlorine analysis that is usually analyzed in most U.S. EPA survey programs, the calibrant mastermix should contain the following target analytes:

| | | |
|---|---|---|
| 1,2,4,5-tetrachlorobenzene | 2,4'DDD | dieldrin |
| 1,2,3,4-tetrachlorobenzene | 4,4'DDD | endrin |
| hexachlorobenzene | Heptachlor | mirex |
| α-HCH | Heptachlor epoxide | endosulfan II |
| β-HCH | Oxychlordane | endosulfan sulfate |
| γ-HCH | α-chlordane PCB66 | PCB8 |
| δ-HCH | PCB101 | PCB18 |
| pentachlorobenzene | PCB105 | PCB52 |
| chlorpyrifos | PCB118 | PCB170 |
| pentachloroanisole | PCB128 | PCB180 |
| 2,4'-DDE | PCB138 | PCB187 |
| 4,4'DDE | PCB153 | PCB195 |
| 2,4,'DDT | γ-chlordane | PCB206 |
| 4,4,'DDT | trans-nonachlor | PCB209 |
| | cis-nonachlor | PCB28 |
| | aldrin | PCB44 |

### 3.3.4. Matrix Spike Standard

The purpose of the matrix spike standard is to determine the efficiency of the recovery of target analyte from an actual sample. To conform to U.S. EPA methods, a matrix spike duplicate should be included in the analytical laboratory's methodology. The matrix spike duplicate is used to determine the precision of matrix recovery. These matrix spike standards can be obtained from a commercial source and are certified/designated for use as standards. The concentration of analytes in the matrix spike solution should be about 10 times greater the concentration of the methods detection limit. The matrix spike standards are added to a sample. The matrix spike solution usually contains the same target analytes found in the working standard solution or a subset of those target analytes.

### 3.3.5. Blanks

Included in the Analysis Laboratory's methodology should be a number of Laboratory Method Blanks. This blank, actually a set of blanks, includes components used for analysis. For example, one blank should include the analysis of the dichloromethane and hexane from the original reservoir. Glassware used during the extraction should also have a blank. The Teflon sample containers, and ever the aluminum foil used to contain some samples should have blanks of their own. To counter possible criticism, the the Hydrosorb wipes should be used to on 'clean' Teflon cutting boards, dissecting instruments, and counter-tops to determine the extent of possible sample preparation contamination.

### 3.3.6. Standard Reference Material

Standard reference material is usually obtained from a government repository or some other certified source whose sample and sample matrix is similar to the samples. This material is processed through the entire extraction method, determined for lipid content and dry weight, and analyzed with the samples usually in the same batch of samples that are loaded onto the analytical instrument.

## 3.4. Quality Control/Quality Assurance Parameters

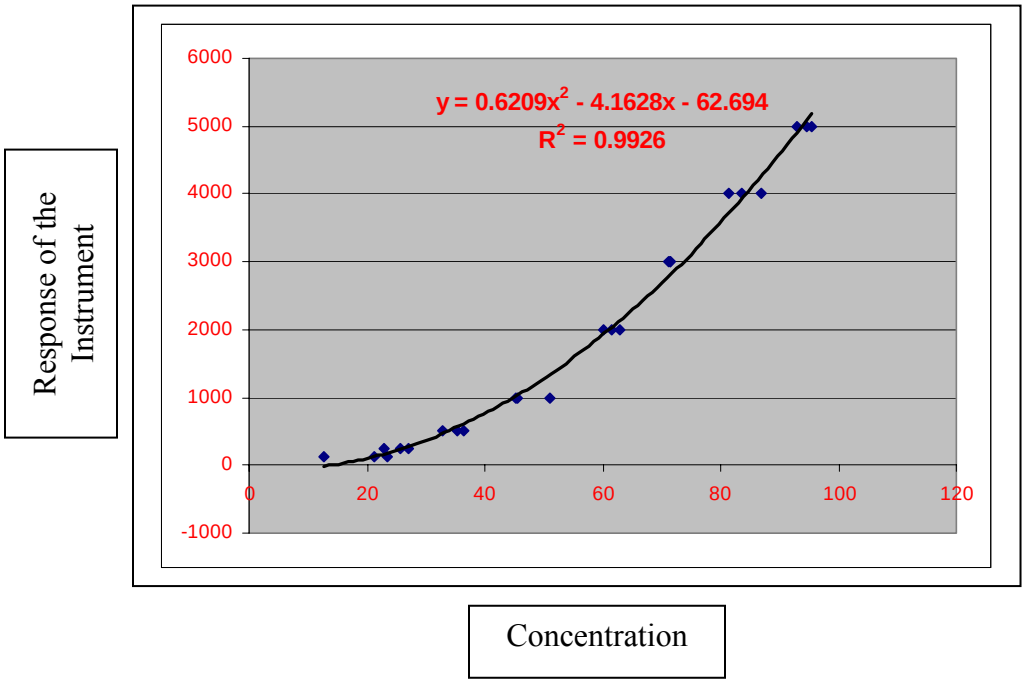
Whatever laboratory is responsible for conducting the contaminant analysis for your samples, it should have a Quality Assurance (QA) program in place that meets the demands of specific Quality Control (QC) criteria. Quality Assurance is a 'management strategy' that meets the demands of the study for a level of confidence in the validity of the data being generated.

As part of the Quality Assurance Program for the analysis laboratory, the laboratory should be able to provide you (on demand) documentation that addresses the following items (which is what expected as part of an US EPA QA/
QC program and is copied almost verbatim from Guidance for Assessing Chemical Contaminant Data for Use In Fish Advisories. Volume 1: Fish Sampling and Analysis - Third Edition appendix I):

1. Detailed descriptions of laboratory procedures for sample receipt, storage, and preparation.
2. Detailed descriptions of the analytical methods used for quantitation of target contaminants, dry weight of sample, and percent lipid determination.
3. Detailed descriptions of methods routinely used to assess data accuracy, precision, and completeness.
4. Detailed descriptions of preventive maintenance procedures for sampling and analysis equipment.
5. Detailed descriptions of calibration procedures for all measurement instruments, including specification of reference materials used for calibration standards and calibration schedules.
6. Detailed descriptions of procedures for internal QC performance and/or systems audits for sampling and analysis programs.
7. Detailed descriptions of procedures for external QA performance and/or systems audits for sampling and analysis programs, including participation in certified QA proficiency testing or interlaboratory comparison programs

Every analysis laboratory should be able to meet five separate QC criteria. They can present their QC in different ways, but you should be able to feel satisfied that they have met these criteria.

1. Demonstration of capability – The laboratory must demonstrate that all of the instruments are in sound working order, that the technicians are competent, and that the lab has a quality assurance plan in place.
2. Laboratory background contamination – the laboratory must demonstrate that benchtop, the sample preparation instruments, solvents, the analytical instruments are free of traces of target analytes. The lab should provide method blank data and nonzero blanks for both sample analysis and analyte percent recoveries.
3. Determining surrogate and targeted analyte recovery – the laboratory must demonstrate that the surrogate and targeted analytes can be recovered within an acceptable range by the methods preparation in that laboratory. It is beyond the scope of this Protocol, but an excellent review for theory and application of surrogate and targeted recovery can be found in Loconto, P.R. (2005) Trace Environmental Quantitative Analysis: Principles, Techniques, and Applications, 2nd edition. CRC Press. Boca Raton. Pp 82-91. What you need to understand from the analytical laboratory is the range of recovery of a target analyte using the laboratory's methods, and what you require to be an acceptable range of %recovery.
4. Experimental calibration and the range of linearity - The analytical laboratory will provide you with data that has quantitative units. There is a method for which they determined concentration of each target analyte. That method includes the addition of known concentrations of a target analyte that is analyzed by the analytical instrument. This data represent two characteristics: (1) the response of the instrument and (2) concentration of the analyte. This data can be represented as a relationship (quadratic relationship).




The correlation coefficient, ***r***, of 0.9990 is the goal to achieve between the instrument response and the amount of analyte. The coefficient of determination, $r^2$, is the measure of the variation of the instrument response (dependent variable) that can be accounted for by the concentration of the target analytes (independent variable). These two coefficients are necessary to determine the range of linearity and the goodness of fit in the calibration of an instrument for measuring the target analytes. The lab should establish what the linear dynamic range of their instrument is for a target analyte, whether the linear range be determined by a first-order least squares regressions or a second order least-squares regression (polynomial), you should understand that for your sample to have a validly determined concentration of a target analyte, the instrument's response for your sample's target analyte must fall within the linear range of the calibration curve. If your sample's target analyte falls outside the linear range of the calibration curve, it produces an increased level of uncertainty in measuring accurately the true concentration of the analyte in that sample.

5. Precision and Accuracy – The laboratory should provide you documentation of the precision of the method/instrument in measuring the target analyte in each sample, and the accuracy of the measurement. Precision can be evaluated by conducting replicate measurements on the same sample for the same target analyte. Most assays are conducted in triplicate so that a meaningful standard deviation can be determined for you sample, as well as determining the %coefficient of variation.

Accuracy can be determined by the use of a known, certified, reference standard. Your sample's response/calculated concentration can be compared to the reference standard, and a standard error determined. The accuracy should be reported as a "% standard error."

%Error=[Xi(unknown) – Xi(known)]/Xi(known)

### 3.5. Reports of contaminant chemistry analysis

Following U.S. EPA suggestions for data reporting of contaminant chemistry analysis, documentation of analytical data for each sample or batch of samples should include the following information:

1. Study identification (e.g., project number, title, phase).
2. Description of the procedure used, including documentation and justification of any deviations from the standard procedure.
3. Method for the detection and quantitation limits for each target analyte.
4. Method for the accuracy and precision for each target analyte.
5. Discussion of any analytical problems and corrective action taken.
6. Sample identification number.
7. Sample weight (wet weight).
8. Lipid content (as percent wet weight).
9. Final dilution volume/extract volume.
10. Date(s) of analysis.
11. Identification of analyst.
12. Identification of instrument used (manufacturer, model number).
13. Chromatograms for each sample analyzed by GC/ECD.
14. Raw data quantitation reports for each sample.
15. Description of all QC samples associated with each sample (e.g., referencematerials, field blanks, rinsate blanks, method blanks, duplicate or replicate samples, spiked samples, laboratory control samples) and results of all QC analyses. QC reports should include quantitation of all target analytes in each blank, recovery assessments for all spiked samples, and replicate samplesummaries. Laboratories should report all surrogate and matrix spike recovery data for each sample; the range of recoveries should be included in any reports using these data.
16. Analyte concentrations with reporting units identified (as ppm or ppb wet weight **and** ppm or ppb lipid weight, to two significant figures unless otherwise justified). **Note:** Reported data should not be recovery-corrected or blank-corrected.
17. Data qualifications (including qualification codes and their definitions, if applicable, and a summary of data limitations).