NATIONAL STATUS AND TRENDS BIOEFFECTS PROGRAM: FIELD METHODS

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BACKGROUND

Environmental quality indicators provide resource managers with information useful to assess coastal condition and scientifically defensible decisions. Since 1984, the National Oceanic and Atmospheric Administration (NOAA), through its National Status and Trends (NS&T) Program, has provided environmental monitoring data on chemical, physical, and biological indicators of coastal environments. The program has two major monitoring components to meet its goals. The Bioeffects Assessments Program evaluates the health of bays, estuaries, and the coastal zone around the nation using the Sediment Quality Triad technique that includes measuring sediment contaminant concentrations, sediment toxicity and benthic community structure. The Mussel Watch Program is responsible for temporal coastal monitoring of contaminant concentrations by quantifying chemicals in bivalve mollusks.

The NS&T Program is committed to providing the highest quality data to meet its statutory and scientific responsibilities. Data, metadata and information products are managed within the guidance protocols and standards set forth by NOAA's Integrated Ocean Observing System (IOOS) and the National Monitoring Network, as recommended by the 2004 Ocean Action Plan. Thus, to meet these data requirements, quality assurance protocols have been an integral part of the NS&T Program since its inception. Documentation of sampling and analytical methods is an essential part of quality assurance practices. A step-by–step summary of the Bioeffects Program's field standard operation procedures (SOP) are presented in this manual.

Chapter 1: INTRODUCTION

NOAA conducts Bioeffects Assessments studies that are customized to the needs of specific geographical areas of concern and uses a variety of techniques that comprise the Sediment Quality Triad Approach (Chapman et al. 1987), a combination of sediment chemistry, benthic infauna characterization and sediment toxicity bioassays. Bioeffects studies are generally a one-time intensive sampling effort that may include both stratified random and targeted site selection.

The goal of the National Status and Trends Bioeffects Assessments Program is to evaluate the magnitude and extent of contamination that impacts habitat conditions which influence biodiversity and distribution of benthic infaunal communities in coastal waters. The objectives are to determine the environmental health of coastal and estuarine areas by characterizing incidence and degree of surficial sediment toxicity; determine the spatial patterns or gradients in chemical contamination and toxicity, if any, and determine the association among measures of sediment contamination, toxicity and the benthic macroinvertebrate community structure.

Critical habitats and food chains supporting many estuarine fish and wildlife species include the benthic environment. Contaminants in the sediments often pose both ecological and human-health risks through degraded habitats, loss of fauna, biomagnification of contaminants in the coastal ecosystem, and human consumption of contaminated fish and wildlife. In many instances, fish consumption advisories are coincident with severely degraded sediments in coastal water bodies. Thus, characterizing sediment quality by describing benthic assemblages and delineating areas of sediment contamination, toxicity and body burdens in higher trophic levels are viewed as important goals of coastal resource management.

Macrobenthic organisms play an important role in the estuarine environment. As major secondary consumers in the estuarine ecosystem, they represent an important link between primary producers and higher trophic levels for both pelagic and detritus-based food webs. Benthic assemblages are composed of diverse taxa with a variety of reproductive modes, feeding guilds, life history characteristics, and physiological tolerances to environmental stressors, both natural and anthropogenic. Benthic assemblages respond to many stressors such as toxic pollution, eutrophication, sediment quality, habitat modification, and seasonal climate changes. Their composition, abundance, and biomass also are influenced by habitat conditions including salinity and sediment type. Benthic community studies have a history of use in regional estuarine monitoring programs and have been proven to serve as an effective indicator for describing the extent and magnitude of pollution impacts in estuarine ecosystems, as well as for assessing the effectiveness of management actions.

Sediment contamination is a major environmental issue because of its potential toxic effects on biological resources. NS&T uses a suite of sediment toxicity tests to assess different modes of contaminant exposure (bulk sediment, sediment porewater, and chemical extracts of contaminants from sediment) to a variety of species (invertebrates, bacteria, and vertebrate cells) and different assessment end-points (i.e., mortality, impaired reproduction, physiological stress, and biomarker response). Typically, the amphipod mortality test, the sea urchin fertilization impairment test, the Microtox test, and, in recent years, a Human Reporter Gene System (HRGS) P450 test are used in each study area. Other tests, based on promising new techniques, full life-cycle tests, and genotoxicity have also been used in some area on a trial

basis or in response to a specific information need. In addition, the Bioeffects Program has initiated the collection of larger and more mobile organisms to assess contaminant exposures and impacts relevant to higher trophic levels and sea food safety.

PLANNING AND PREPARATION

Planning

The level of advanced planning varies with study locations, experience and training of personnel, resource requirements, vessel platform and who has jurisdictional authority over where the study area will be. Given that the Bioeffects managers in coordination with local principal investigators and collaborators have agreed upon mission goals, a plan of targeted or a stratified random design should be made with a list of primary and alternative sites to be visited. Also included in the plan are the type and quantity of samples to be collected. The following items need to be addressed:

1) Submit applications for sampling permits early as this process may take several months;

2) Identify transportation required to navigate to the sites, and any institutional policies for their use (cars, ferries, boats, bush planes);

3) Identify field crew personnel, skills required (e.g., SCUBA), and training needs (safety and equipment use);

4) Identify sampling equipment and supply needs – including their sources, procurement, and shipment;

5) Identify and procure chemicals (for cleaning and sample preservation) and arrange for their proper storage, transportation, use, and disposal;

6) Prepare a logistics plan that takes into account unique site visit requirements: permissions, access and navigation, equipment, tides, depth, transit timeand currents.

PLANNING RESOURCES

- NOAA Tides and Current Predictions (http://tidesandcurrents.noaa.gov/)
- NOAA Office of Coast Survey Raster Charts (http://www.charts.noaa.gov/RNCs/RNCs.shtml)
- NOAA Weather (http://weather.gov)
- NOAA Marine Weather (http://www.nws.noaa.gov/om/marine/home.htm)
- NOAA Dive Program (http://www.ndc.noaa.gov/)
- List of NOAA Bioeffect Staff and Laboratory Contacts (Appendix 1)

Field Equipment and Materials

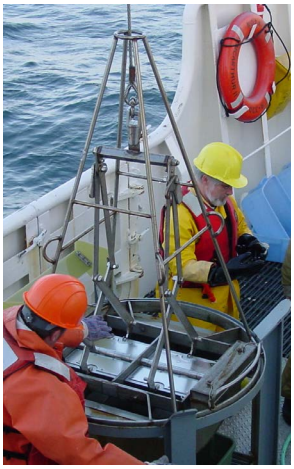
The field team lead must develop a check list (Appendix 2) of sampling equipment and materials prior to the start of the day of the field trip and assure that proper sample containers and labeling materials are accounted for. The list must include field measurement equipment and materials listed below. The field team must also assure that all field equipment is in good working condition and have back ups for critical equipment.

Logistics: GPS, roadmaps and nautical charts, transportation (boat, automobile), target sampling list.

Sediment equipment: PONAR or Van Veen sampler, Teflon coated titanium or stainless steel scoop, glass jars for chemical samples, whirlpack bags, mixing bucket and liners, mixing drill, plastic and /or glass containers for bioassay samples, labels, electrical tape, acetone, ice, formalin, coolers, cleaning gear, notebooks, datasheets, pens, camera, YSI or multimeter, gloves.

Shipping equipment: Prelabeled overnight shipping labels, box labels, tape (strapping, duct), bubble wrap bags, chain of custody sheets, padding, dry ice or freezer packs, vermiculite.

Examples of Standard Sediment Grabs



Dual Young-modified Van Veen grab;
Stainless steel construction, for collecting in offshore environments.

All field equipment must be checked and calibrated daily or as required by the manufacturer. All field-QC check procedures must be appropriately documented including dates and name of the person conducting the procedures.

Instrument Calibration

All field equipment must be checked and calibrated daily or as required by the manufacturer. All field-QC check procedures must be appropriately documented including dates and name of the person conducting the procedures.

Personnel Training and Safety

The Bioeffects Program conducts sediment quality studies all around the US and its territories from Alaska to the Caribbean and Pacific Islands. Each region has unique conditions that require consideration when preparing for the project. All field activities associated with Bioeffect studies are conducted aboard a boat, thus it is beyond the scope of this document to describe each consideration regarding personnel safety for vessels ranging from small boats to ocean-going ships . It is the responsibility of the field team to acquire boat safety and local knowledge required for a safe and successful mission. Here is a brief list of some issues to consider in training personnel and planning field activities:

- Situational awareness of weather and the environment;
- Boat safety as recommended by NOAA ;
- Local knowledge of surf, tides and currents;
- Navigation skills with GPS, nautical charts and land maps;
- Field crew of three or more, each with knowledge and stamina to contribute to the team;
- Appropriate form of electronic communication (cell phone, VHF radio, Sat phone);
- Ability to respond to first aid emergencies;
- Wear appropriate clothing for thermal (cold or warm) and water protection. Review coldwater safety prior to entering the field;
- Appropriate safety equipment and knowledge to use it;
- Appropriate sampling equipment and knowledge to use it (sediment grabs, oyster dredge, water quality meter, etc.);
- Understanding of the sample collection and shipping procedures.

Coastal environments can be dangerous and unpredictable; exercise due caution. Field teams should use good judgment and not risk their personal safety when conditions pose undue risk. Abandon the site and return when conditions improve.

Deviations from the SOP are sometimes necessary but approval from the Program Manager should be obtained prior to sampling. If prior approval is not possible, notification of Program staff should be done as soon as possible. In every case, changes in sampling procedures or location must be clearly documented by the field crew. The following section lists tasks that should be accomplished prior to entering the field.

SITE SELECTION PRECEDURE

Location and site selection

Bioeffects assessments are usually conducted in embayments or estuaries based on a variety of parameters, including: (1) the likelihood of adverse biological effects of contamination based on state and local environmental data; and (2) collaboration with other Federal, state, and local agencies, and academic institutions; (3) concentrations of contamination in oysters or mussels as determined by NOAA's NS&T Mussel Watch Program which points to the need for a more comprehensive localized study. However, assessments have also been conducted to establish background data on coastal areas, embayments and estuaries of particular interest.

One of the principles of the bioeffect assessment is to apply the same suite of tests synoptically to all areas so that comparisons can be made without the confounding interference of using different methods in different areas. Thus, comparison of spatial extent of impact between areas is possible even if the areas are not contiguous.

Stratified Random Site Selection

Once selected, the study location is subdivided into strata. Strata boundaries are usually established in consultation with regional scientists and resource managers, and are based on bathymetric, hydrographic, and regional environmental considerations, and previous studies detailing geochemical composition, sediment grain size distribution, organic carbon maps, etc. Sampling sites within each stratum are established based on stratified-random design, which statistically allows equating sites to determine the spatial extent of sediment toxicity and or chemical contamination in the study location. Creation of the random stratified sample sites is done using ArcGIS and the GIS tool Geospatial Modeling Environment (GME), which is based on stochastic algorithms. Once each stratum is identified and the total number of samples to be collected decided GME generates the requested number of points for each strata separately. A 50m buffer between sample sites is used to avoid placing random sample locations on top of each other and to allow for GPS variance on site. The approach combines the strengths of a stratified design with the random-probabilistic selection of sampling locations, allowing the data generated within each stratum to be attributed to the dimensions of that stratum with a quantifiable degree of confidence (Heimbuch et al., 1995). Additionally, randomly selected alternate sites are also selected for each primary sampling site. Both primary and alternate sampling locations are generated by GME simultaneously. The first generated locations always are designated as the primary sites followed sequentially by the alternate sites. For example, if there are five primary sites and ten alternates GME will generate fifteen random sample locations. The first five random sites are designated as the primary sites and the last ten random sites as the alternates. In instances where the primary site cannot be sampled due to non-accessibility or an unsuitable substratum, the first alternate site is sampled, which in the above example would be the tenth generated location. Sampling of alternate sites continues sequentially if necessary thereby maintaining the statistical random-stratified design. As a result of sequential sampling of alternates, they may or may not be the nearest alternate site to the abandoned primary site.

Station Inaccessibility

On occasion a set of site coordinates that has been randomly generated cannot be sampled. Reasons may include: the site is too shallow; location has no depositional sediments; unforeseen circumstances such as an accident or oil spill; or there is no dredging or anchoring allowed in the area. If a station is deemed inaccessible, a notation should be made in the log. The alternate site, if any, should be sampled. Under no circumstances should stations be moved for the convenience of sampling. It is important to maintain this procedure for selecting the alternative site and to record the reasons for inaccessibility of the primary and any subsequent alternate sites on the log sheets.

Alternative Approaches

Traditionally, NS&T Bioeffects studies have used the stratified random sampling design. This approach is widely used in a variety of programs (e.g. EPA Environmental Monitoring and Assessment Program, Southern California Water Research Project, Central Coast Long-term Environmental Assessment Network (California), Puget Sound Ambient Monitoring Program). Indeed some aspects of these programs were derived from, or were developed in tandem with NOAA NS&T.

There are many other approaches to sampling depending on the specific objectives of the study (Gilbert, 1987). Of fundamental importance is the concept of the target population and the sampled population. The target population is the set of all possible sample locations which the study intends to characterize. The sampled population is that set of locations actually sampled and used to derive inferences about the target population. The size of the sampled population will be determined by available resources and a-priori assumptions about variability in the study area. With limited budgets, the density of sample points will be a trade-off between the size of the area to be characterized, variation, and the desired level of precision. Stratification is an approach to reduce variation by grouping subsets of sample locations into presumed similar habitat types, which will likely have similar physicochemical characteristics, which in turn will drive contaminant accumulation patterns and benthic infaunal communities.

In general, a minimum of 3 samples must be taken in each stratum in order to make statistical inferences. Otherwise, the analyst cannot do more than simply compare gross counts, be it a chemical or biological measurement. There are other sample designs that could be used depending on the objectives of the study, including simple random sampling, systematic sampling, grid sampling, etc. These sampling design approaches may be more useful where known hot spots occur, or when the objective is to examine the extent of a gradient from a known source for example. The original intent of the Bioeffects Program which was the ability to infer the relative spatial proportion of impacted habitat between areas may be lost however. The fundamental requirement for a triad of measurers of chemical concentration, effect (e.g. toxicity) measurement, and biological condition remains the same. In recent years, the Bioeffects Program has begun to expand the spectrum of potential endpoints to define effects and biological condition. In coral reef environments for example, histological and disease endpoints may be used to assess effects. Similarly, measurements of nutrients and sedimentation rates are included with conventional toxic contaminant analyses as these are significant irritants to coral. The Bioeffects approach is also being expanded to include tissue body burdens of resident organisms at higher trophic levels. This data is relevant to exposure and health of organisms, and also is directly applicable to human health issues with regard to sea food consumption. This has proved to be of particular importance in areas such as Alaska where a subsistence lifestyle is not uncommon.

Chapter 2 Sediment Sampling For Contaminant Toxicity and Benthic Community Analyses

Overview

Benthic habitat quality assessment is the core of NS&T Bioeffects assessments. This includes not only detailed sediment chemistry, toxicity, and physical nature of sediments, but also the water quality and physical setting of the habitat (depth, currents, temperature regime, oxygen, etc), all of which will determine the nature and resilience of the biological community present. This chapter addresses the collection of sediment quality triad (SQT) samples and ancillary measurements used to assess habitat condition. Only by understanding the underlying drivers of benthic community distributions can the impact(s) of contamination be identified.

1.0 Sediment Quality Triad Sampling

Sediment samples are collected using cleaned (washed, acid rinsed, deionized water (DI) rinsed, acetone rinsed, DI rinsed) stainless steel 0.1m2 Young-modified Van Veen or a 0.04m2 Ponar grab sampler which is to be cleaned between stations. Once retrieved, the sediment must be inspected for surface disturbance (Figure 1). If the sediment is very soft or includes a floc layer, and has been squeezed out from the top cover of the sampler, the surface layer has been lost and another sample cast is required. A slower descent rate and/or flotation added to the grab frame and/or a ‘snowshoe’ on the bottom of the frame may be required to prevent the sampler from sinking too far into the bottom. When an acceptable sample is obtained (Figure 2), the surface should be photographed to document color and texture. A stainless steel scoop is used to carefully remove the top 2-3 cm of sediment materials. Multiple grab samples may be taken and composited into a bucket with an acetone and DI-rinsed high-density polyethylene (HDPE) liner, until sufficient volume (3-4L) of sediment for all the toxicity bioassays and chemical analysis is collected. The bucket liners are not to be reused between sampling sites. After thoroughly homogenizing the sediment samples in the field with an acetone and DI-rinsed, stainless steel mixer, the composite samples are subdivided for distribution to various testing laboratories. Samples for chemical analyses must be stored in 250 ml pre-cleaned glass jars with Teflon liners. A subsample for grain size analysis is stored in a Whirl-pak bag. Subsamples for toxicity testing are to be stored in test-specific containers. For amphipod bioassays, samples are stored in 1 L polyethylene jars with Teflon lined lids. For pore water bioassays, samples are stored in 1 gallon polyethylene jars with Teflon lined lids. For sediment extract bioassays (P450, Microtox), samples are stored in 125 ml pre-cleaned glass jars with Teflon lined lids. Additional subsamples may be retained for specialized bioassays. Following sampling, the grab and scoop must be thoroughly scrubbed to remove all traces of sediment and washed down with site water.

A separate sample(s) is taken for benthic community analysis with a 0.1m2 Van Veen grab sampler or a 0.04m2 PONAR grab sampler. The entire content of acceptable grab (at least 5 cm deep) is sieved on site through 0.5mm mesh. In coarse sediments, nested sieves of 1.0 mm and 0.5 mm may be necessary to reduce clogging of the screens and damage to the organisms. All organisms are retained in plastic containers and preserved in buffered 10 % formalin containing Rose Bengal stain and sodium borate buffer. At each station, additional subsamples may be taken for ancillary measurements such as meiofauna, foraminifera, comet bioassay, etc. These samples are stored in plastic or glass containers.

The following data and information should be recorded at each station: stratum, site, alternate (if applicable), date, depth, time, latitude, longitude, # grabs taken, a filled out checklist of samples processed, written description of each sampling site including digital color photographs of the site, a physical description of sediment characteristics (texture, color, odor, benthos sheen and photographs of the undisturbed sediment. Sediment and water samples from each station are to be properly labeled and preserved on ice or frozen until shipped. Benthos and tissue samples are to be properly labeled and preserved in fixative or frozen until shipped.

1.1 Field Equipment and Materials

A thorough list of equipment/supplies needed are detailed in Appendix 2. The field team lead must develop a check-list of sampling equipment and materials prior to the start of each field day and assure that proper sampling gear, containers and labeling materials are available. The list must include field measurement equipment and materials listed below. The field team must also assure that all field equipment is in good working condition and have back-ups for critical equipment.

Logistics: GPS, roadmaps and nautical charts, transportation (boat, automobile), target sampling list.

Sediment equipment: PONAR and/or Van Veen sampler, Teflon coated titanium or stainless steel scoop, glass jars for chemical samples, whirlpac bags, mixing bucket and liners, mixing drill, plastic and/or glass containers for bioassay samples, benthos sieves, benthos containers, sample container labels, electrical tape, clear tape, acetone, ice, formalin, coolers, cleaning gear, notebooks, datasheets, pens, digital camera , gloves.

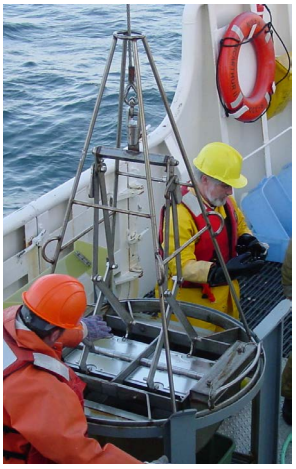
Water Quality equipment: YSI or Hydrolab multimeter, secchi disk or turbidity meter, Nisken bottle/CTD/Rosette sampler, water sample bottles.

Examples of Standard Sediment Grabs

Ponar Grab Sampler: Stainless steel construction, 6” x 6” jaws (0.04 m sq), stainless steel doors on top, for hand collection of samples, with or without davit.

Young-modified Van Veen grab; Stainless steel construction, jaws 12"' x 12" (0.1m sq), stainless steel doors on top, for collection with davit or A frame and winch, with or without frame.

Dual Young-modified Van Veen grab; Stainless steel construction, for collecting in offshore environments.



Examples of Standard Water Column Samplers

Example Niskin water sampling bottle for taking bottom water samples for water quality measurements. Depending on the construction materials of the bottle, it may or may not be appropriate for water chemistry samples (e.g. PAHs)

A typical CTD (Conductivity/Temperature/Depth) meter for profiling water quality parameters through the water column. Additional sensors may be mounted such as fluorometer, dissolved oxygen, etc.

A rosette for sampling the water column at specific depths in deep-water stations. Rosettes typically include a CTD located in the center of the array.

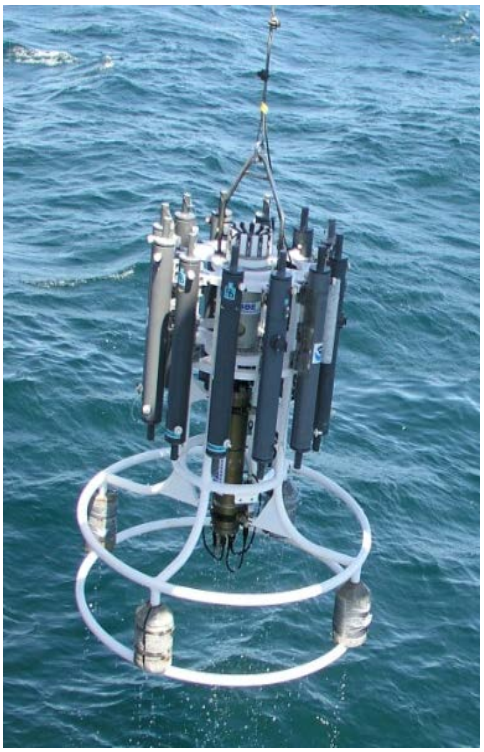


Figure 1. Example of very soft sediment that has extruded out of the grab from under the door covers. The surface layer has been partially lost.

Figure 2. Examples of a grabs in which the surface layer is intact. The brown oxidized surface layer is present as well as epbenthic animals.

For each day, the field team will need:

a. Log book, data sheet & pens
b. Hand held GPS
c. HDPE bucket (with lid) for homogenization of sample
d. Mixing drill
e. Water quality measurement instruments
f. Benthos Sieve
g. Per site needs:
i. Pre-cleaned glass jar (250mL) for organics
ii. Pre-cleaned glass jar (250mL) for metals
iii. Whirl-pak bag for grain size sample
iv. Pre-cleaned scoop
v. Pre-cleaned bucket liner
vi. Pre-cleaned sample stirrer

vii. Clean gloves
viii. Syringe & siphon hose
ix. Plastic ruler
x. Paper towels, sponges
xi. Bioassay sample containers
xii. Benthos sample container(s)
h. Tub for discarded sediment

1.2 Methods for Sediment Quality Triad Field Sampling
Order of sample collection in the field

1. It is preferable to collect water chemistry data first to avoid introducing bottom sediment or other material into the water column.
2. Sediment grabs for benthic infauna characterization should be collected following the water quality measurements.
3. Sediment sample for chemistry and toxicity are to be collected last. This allows sieving the benthos samples to begin while the samples for chemistry and toxicity testing are being collected.

1.2.1 WATER QUALITY MEASUREMENT

1.2.1.1 Shallow Water

In shallow water measurements are to be taken at the surface (0.5 m below the surface) and bottom (0.5 m above the sediment) at each site. Water data includes clarity, temperature, salinity and dissolved oxygen. . Use a water quality meter (YSI, r Hydro lab, or equivalent) to measure and record the following physical parameter of the water at each site:

- turbidity
- Depth (m)
- Temperature (degree C)
- Salinity (ppt)
- DO (mg/L)

A secchi disc should be used to measure water clarity if a turbidity meter is not available. Water depth is based on actual measurements at the time of the field collections. Depth to bottom should be determined by depth sounder, or weighted line. Depth measurements are to be reported in increments of meters. Time of measurement should also be noted, especially in areas where tidal range is large. When using a hull mounted depth sounder, it is important to know at what depth the device is mounted under the waterline to make later adjustments in actual (vs measured) depth.

1.2.1.2. Deep Water

In deeper water, surface measurements should be taken as described above. A Niskin bottle (below left) should be deployed to sample bottom water. If available, a CTD (right) cast can be deployed to record a profile of the water column. In coastal waters, sampling off an ocean-going vessel, a rosette including a CTD and water sample bottles may be deployed (above right).

1.2.2 Benthic Infauna Sampling
1. Deploy grab sampler (special techniques may be necessary in the presence of surface contamination, e.g. oil, to avoid contamination at the air: water interface).
Lower sampler through the water column (in shallow water [<5 m] lower at about 1m/sec) in order to minimize disturbance of surficial sediments. In very soft sediment or where a flock layer is present, a life ring may be attached to the top of the frame, and/or a 'snowshoe' on the bottom to minimize penetration into the sediment. If the sediment is hard or very sandy, the sampler may be lowered faster. If necessary, weights may be added to the sampler frame to enhance penetration into the bottom. 'Working' the line up and down may help to dig the jaws into the sediment as the sampler closes.

2. Record on data sheet (Appendix 3):
a. SAMPLE STRATUM/ZONE

b. SAMPLE SITE & ALTERNATE (If applicable)
c. DATE
d. TIME when sampler hit bottom
e. GPS LOCATION (Latitude and longitude in decimal degrees to 4 decimals)
f. WATER DEPTH (m)
g. Record station condition (floating trash, surface oil sheens, buoy markers, etc.)

3. Recover Grab

a. Watch as grab surfaces and note draining water & color for evidence of sediment washout. Set on deck and open lid. Evaluate whether it is intact and has sufficient sediment for sampling. An acceptable sample condition is characterized by a relatively even surface with minimal disturbance and little or no leakage of the overlying water. Heavily canted samples are unacceptable. Samples with a large amount of "humping" along the midline of the grab, which indicates washing of the sample during retrieval, are also unacceptable.
b. A 'keeper' is a sample with level, intact sediment over the entire area and a sediment depth at the center of at least 5 cm (roughly 2 inches) (Fig 3). If it is not a keeper, dispose of the sediment, rewash sampler, scrub any remaining sediment from sides and corners, and redeploy.
c. If the sample is acceptable, record depth of sediment sample in the center of the grab. Also record any evidence of sample washout, uneven collection, epifauna, etc.
d. Empty entire contents of sample into a holding container for temporary storage prior to sieving. Collect all sediment, washing sampler with water to retain all contents.
e. Repeat deployment if collecting replicate samples

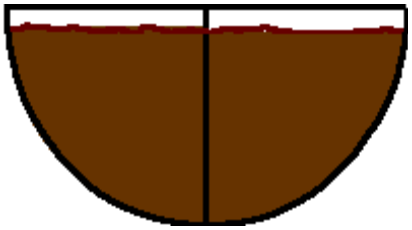
4. Record observations in sample log:

a. Depth of sample collected at center of grab.
b. Texture of sediment.
c. Color of sediment
d. Odor (if present)
e. Any visible benthos
f. Photograph sediment surface

11. Empty sediment from grab & hose down sampler, bucket, and deck in preparation for next station.

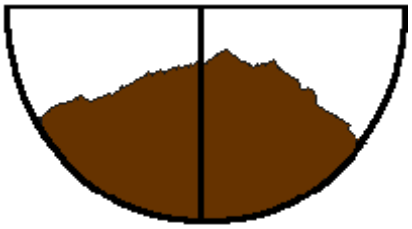
12. RECORD SAMPLE TEAM (legibly)

13. INITIAL DATA SHEET (legibly)

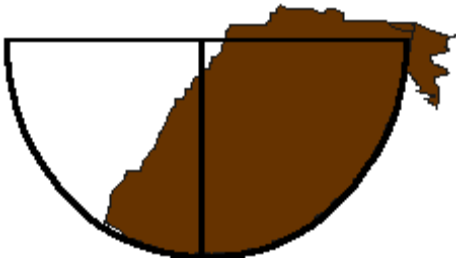


Acceptable

Level, intact sediment over the entire area of the



Unacceptable
Washed out



Unacceptable

Partially filled, sample extending through top

Unacceptable
Rock, shell, or other debris caugh in jaws

Figure 3. Criteria for acceptability vs. unacceptability of sediment samples

1.2.3 Sediment Sampling for Chemical and Toxicity Testing
The following specific operations should be conducted at each site;

1. Prep & label all sample containers ahead of deployment (Appendix 4)

a. All sample containers should be labeled prior to sampler deployment. Sets of containers may be prepared in advance for sampling multiple sites off small vessels.
b. Jar caps are labeled "NOAA, Project, Stratum-Station, date" with a sharpie in addition to pre-printed paper label on side
c. The standard chemistry container inventory includes:
    i. Two pre-cleaned, glass, 250mL jars for organics/TOC and metals
    ii. Whirl-pak sample container for grain size
    iii. Additional 250mL jars may be necessary for additional analyses (e.g. dioxin, PBDES)
d. The standard toxicity bioassay container inventory includes:
    i. Pre-cleaned, glass, 125 or 250mL jars for organic extracts (P450, Microtox)
    ii. One 1gal plastic jar for pore water extraction
    iii. One 1L plastic bottle for whole sediment amphipod bioassay

2. Clean all sampling tools prior to sampler deployment

a. Rinse with site water, followed by acetone, and then distilled water
b. Tools that require cleaning before each station include:
    i. The grab sampler
    ii. Teflon-coated or stainless-steel scoop for sampling sediment
    iii. Siphon hose & syringes used for removing overlying water inside grab (if necessary)
    iv. Sample stirrers

3. Deploy grab sampler (special techniques may be necessary in the presence of surface contamination, e.g. oil, to avoid contamination at the air: water interface).

Lower sampler through the water column (in shallow water [<5 m] lower at about 1m/sec) in order to minimize disturbance of surficial sediments. In very soft sediment or where a flock layer is present, a life ring may be attached to the top of the frame, and/or a 'snowshoe' on the bottom to minimize penetration into the sediment. If the sediment is hard or very sandy, the sampler may be lowered faster. If necessary, weights may be added to the sampler frame to enhance penetration into the bottom. 'Working' the line up and down may help to dig the jaws into the sediment as the sampler closes.

4. Record in Sample Log:
a. SAMPLE STRATUM/ZONE
b. SAMPLE SITE & ALTERNATE (If applicable)
c. DATE
d. TIME when sampler hit bottom
e. GPS LOCATION (Latitude and longitude in decimal degrees to 4 decimals)
f. WATER DEPTH (m)
g. Record station condition (floating trash, surface oil sheens, buoy markers, etc.)

5. Recover Grab
Watch as grab surfaces and note draining water & color for evidence of sediment washout. Set 'gently' on deck and open lid. Evaluate whether it is intact and has sufficient sediment for sampling (Figure 2). An acceptable sample condition is characterized by a relatively even surface with minimal disturbance and little or no leakage of the overlying water. Heavily canted samples are unacceptable. Samples with a large amount of "humping" along the midline of the grab, which indicates washing of the sample during retrieval, are also unacceptable.

6. Record observations in sample log:
a. Texture of sediment.
b. Color of sediment
c. Odor (if present)
d. Any visible benthos
e. Photograph sediment surface (Appendix 5)

7. Collect sediment samples using tools, containers, and supplies itemized above.
   a. Put on plastic gloves (for protection of both sample and personnel*)
   b. If necessary, remove overlying water using a syringe and plastic tygon tubing, being careful to leave as much of the surface flocculent layer as possible
   c. Collect the top 2-3 cm of sediment with scoop
   d. Place in a pre-cleaned container, enough sample to accommodate all analyses
   e. Fill the glass, 250mL pre-cleaned, labeled jar(s) about 3/4 full
   f. Add about 50g of sediment to the texture bag
   g. Record all samples collected on data sheet

   * Refer to NIOSH guide to chemical hazards and manufacturer specifications. Glove(s) material should be resistant to expected contaminants.

8. When collecting sediment for both chemical analyses and toxicity bioassays, multiple samples will be composited.

a. Rinse a 5 gal bucket liner with acetone and DI water, place in a 5 gal HDPE bucket and cover with lid
b. Collect 2-3 cm sample with scoop and place in bucket liner
c. Store scoop in the bucket with the sample
d. REPLACE LID
e. Dispose of unused sediment remaining in grab sampler. Wash sampler with site water and remove all traces of sediment, scrub if necessary. If circumstances warrant, additional cleaning steps may be necessary. For example, if spilled oil is present on the water surface, hexane washes may be necessary to clean the equipment.
f. Redeploy sampler as above
g. Record lat. & long. coordinates in comments section of data sheet of each successive grab
h. Repeat sediment collection and redeployment of sampler as before, until sufficient sediment volume has been collected
i. Rinse sediment mixer with acetone and DI water
j. Thoroughly homogenize sediment composite in the bucket, being careful to mix in sediment from the bottom and corners of the bucket liner
k. Using sample scoop, subsample sediment into appropriate containers for toxicity testing and chemical analyses
l. Dispose of unused sediment and bucket liner

9. Store samples
a. Check that sample log entries are complete
b. Check that all sample jars are labeled with stratum, station, and date
c. Cover jar paper labels with clear tape
d. Seal chemical jar lids with electrical tape
e. Place jars in bubble bags - DO NOT seal bags
f. Samples to be refrigerated: texture samples (Ziplocs/Whirl-paks), bioassay samples
g. Samples to be frozen: organics, metals, P450/Microtox bioassay , (250mL glass jars, in unsealed bubble bags, laid on side in freezer)

10. Add comments – even if the station is abandoned for some reason, record all activities and whatever measurements are taken for future reference.

11. Store data sheet in data book in lab

12. Keep grab sampler and sediment scoop clean when on deck

a. Cover during long transits to avoid airborne dust and engine fumes
b. Scrub sampler and hose down immediately before use
c. Rinse inside surfaces of sampler with acetone and DI water immediately prior to deployment at the next station

1.2.4 Benthic Infauna Processing
1. Sample processing should begin as soon as possible after the grab has been collected. Make sure holding containers are labeled with station identification number, and replicate number. If possible, pre-filter the rinse water in order to prevent introduction of water column organisms.

2. Transfer sample into the 0.5 mm mesh sieve. If sample is sandy or dense clay, sieve in stages by transferring only a portion of the sample at a time to avoid crushing organisms. Repeat until the entire sample has been sieved. In coarse sediments, it may be necessary to pre-sieve with a 1mm mesh sieve.

3. GENTLY rinse the contents - washing away sediments and leaving organisms, detritus, sand particles, and pebbles and shells larger than 0.5 mm. Remember, rinse gently... rinse gently... rinse gently... avoid direct, heavy jets of water. Remove large pieces of shell, gravel etc. by hand to avoid damaging remaining organisms.

4. GENTLY backwash retained organisms from the sieve into a plastic tub. Be sure to remove small organisms from the seams of the sieve bucket and those clinging to the mesh. If necessary, decant excess water from tub over the sieve bucket.

5. Label sample container(s)
All sample container labels should Include:

- Project
- Stratum/zone
- Station
- Replicate
- Date
- Container # of # if more than one per station
- A paper label containing all the above information in pencil should be included inside each container.

6. GENTLY pour the contents of the tub into a labeled sample container. If the quantity of sample exceeds the capacity of the container (so much in it organisms may be crushed by the weight), place the remainder of the sample in a second (or third) container [fully labeled as above]. The samples will likely be bounced and subject to settling during shipment.

7. Enough formalin should be added so that it covers all organism [seawater should be added to fill remainder of container]. Containers should be near full to help prevent agitation of the fixed organisms and also the possibility of stranding organisms on the sides of the container during shipment. If the samples contain significant quantities of coarse sand, add enough formalin to cover the sediment. Gently rotate the sample to mix the formalin throughout the sediment.

8. Store the containers in a cooler in a secure, well-ventilated area, out of direct sun

9. Thoroughly rinse, brush, and backwash the tubs and sieve. Don't be gentle.

1.2.5. SAMPLE HOLDING AND SHIPPING CONDITIONS

All samples of all types must be meticulously inventoried immediately prior to shipment. This is to ensure a complete record of all samples is generated and to complete an accurate chain of custody log of samples (Appendix 6). Following inventory, the bubble bags for each of the contaminant sample jars can be sealed and the jars carefully packed and padded for shipping.

Sample Type	Holding Conditions in the Field	Holding Conditions on ship/Laboratory	Shipping
Benthos	Preserved in 10% buffered Formalin with Rose Bengal stain	Preserved in 10% buffered Formalin with Rose Bengal stain	End of cruise
Sediment Grain Size	Cooler with water ice	Refrigerator	End of cruise w/ blue ice
Organics/Total Organic Carbon	Cooler with water ice	Freeze	End of cruise w/ dry ice
Metals/	Cooler with water ice	Freeze	End of cruise w/ dry ice
P450 Bioassay	Cooler with water ice	Freeze	Every 4-5 days w/ blue ice
Amphipod Bioassay	Cooler with water ice	Refrigerator	Every 4-5 days w/ blue ice
Porewater Bioassay	Cooler with water ice	Refrigerator	Every 4-5 days w/ blue ice
MircroTox Bioassay	Cooler with water ice	Freeze	Every 4-5 days w/ blue ice

1.2.5.1. Packaging and Shipping of Benthic Samples

Samples for benthic community analysis are preserved in buffered formalin containing Rose Bengal. These samples are in 250-1000ml plastic containers with tight fitting lids and sealed with electrical tape. Since these samples are preserved, there is no need to refrigerate or pack in ice. However, they should be kept in as cool an environment as possible and out of direct sun to minimize vapor release.

Before transport:

1. Check each container lid to assure that it is taped.
2. Place each container individually or as a group in a zip-lock bag (in case of leakage).
3. Place 'bagged' containers into shipping container (i.e., Styrofoam box, cooler, or any protected type shipping container). The shipping container will be double lined with heavy duty plastic trash bags - fill voids in trash bags with vermiculite, and around bags in container with vermiculite. Seal the trash bag liners. Add vermiculite to shipping container to cover liner bags.
4. Include Chief Scientist's signed original of the Sample Shipping List (page 19) inside each container [place in Ziploc bag taped to inside of container lid].
5. Samples should then be shipped via UPS Standard Second Day Service (NOTE: avoid weekend delivery).

   Samples will be shipped to:
   Contractor Contact
   Contractor Address (street address, not PO address)
   Contractor telephone
   Contractor Fax

Upon shipping samples, call or email the above contact person to provide UPS tracking numbers.

NOTE: If there is a 10% or greater concentration of Formaldehyde in the samples, then ***Special Packaging for Shipment is Required***. If unsure about this or, if other chemicals are being shipped, then call UPS at 1-800-742-5877 and ask to speak to a Dangerous Goods Specialist. Current UPS limits can be found at http://www.ups.com/media/en/acceptedtable_jan_2011.pdf.

1.2.5.2. Packaging and Shipping of Metals, Organic/TOC, Samples

Samples for contaminant analyses have been frozen, except for the grain size samples, and shipment with dry ice is preferred, otherwise use frozen blue ice packs. Avoid the use of soft freezer packs as they will eventually leak. Do not use water ice. The sample jars should be sealed individually into bubble bags prior to shipping. Shipping occurs at the end of the sampling cruise.

Before transport:

1. Check each container lid to assure that it is taped with the labeling clearly legible on the lids and sides of the jars. Verify the lids and labels match.

2. Place each bottle individually in a bubble bag to prevent breakage of the jars and seal the bag.

3. Put dry ice in the bottom of a cooler, place cardboard on top of the ice

4. Place the "bagged" jars into the cooler, leaving room for an additional cardboard/dry ice layer. The jars may be layered with foam or placed in the original shipping boxes they came in.

5. Include Chief Scientist's signed original of the Chain of Custody Sheet inside each cooler [place in Ziploc bag and tape to the inside lid]. A copy of the custody sheet should be made prior to shipment

6. Samples should then be shipped via UPS Priority Overnight Service (NOTE: DO NOT ship on a Friday).

NOTE: The UPS shipping label has to indicate whether the shipment contains dry ice. Dry ice may or may not be available in remote areas. Upon shipping samples, call or email the above contact person to provide tracking air bill numbers.

1.2.5.3. Packaging and Shipping of P450 RGS/Microtox Toxicity Samples

Samples for P450 and Microtox bioassays have been frozen, and shipment with dry ice is preferred, otherwise use frozen blue ice packs. Avoid the use of soft freezer packs as they will eventually leak. Do not use water ice. The sample jars and should be sealed individually into bubble bags prior to shipping. Shipping may occur during or at the end of the sampling cruise.

Before transport:

1. Check each container lid to assure that it is taped with the labeling clearly legible on the lids and sides of the jars. Verify the lids and labels match.

2. Place each P450/Microtox¨ jar individually in a bubble bag to prevent breakage of the jars and seal the bag.

3. Put dry ice in the bottom of a cooler, place cardboard on top of the ice

4. Place the "bagged" jars into the cooler, leaving room for an additional cardboard/dry ice layer. The jars may be layered with foam or placed in the original shipping boxes they came in.

5. Include Chief Scientist's signed original of the Custody Sheet inside each cooler [place in Ziploc bag and tape to the inside lid]. A copy of the custody sheet should be made prior to shipment

6. Samples should then be shipped via UPS Overnight Service (NOTE: DO NOT ship on a Friday).

NOTE: Upon shipping samples, call the above contact person to provide UPS tracking air bill numbers.

1.2.5.4. Packaging and Shipping of Amphipod and Porewater Toxicity Samples

Samples for these toxicity bioassays have been kept at 4oC. These samples are in either one gallon plastic containers or 1 L Nalgene jars and should be shipped with sufficient amount of blue ice to maintain temperature during shipping. Shipping occurs every 4 or 5 days of the sampling cruise if possible

Before transport:

1. Check each container lid to assure that it is taped with the labeling clearly legible on the lids and sides of the jars. Verify the lids and labels match.

2. Place containers into a cooler

3. In between these containers place blue ice packs

4. Place blue ice on top of the sample containers and in any available space to prevent shifting during shipping

5. Include Chief Scientist's signed original of the Custody Sheet inside each cooler [place in Ziploc bag and tape to the inside lid]. A copy of the custody sheet should be made prior to shipment

6. Samples should then be shipped via UPS Overnight Service (NOTE: DO NOT ship on a Friday).

NOTE: Upon shipping samples, call the above contact person to provide UPS air bill numbers.

1.2.5.5. Packaging and Shipping of Grain Size Samples

Samples for grain size analyses have been refrigerated, and shipment with blue ice pack is preferred. Avoid the use of soft freezer packs as they will eventually leak. Do not use water ice. Shipping occurs at the end of the sampling cruise.

Before transport:

1. Check each bag to assure that it is taped with the labeling clearly legible on the side of the bag.

2. Put a blue ice pack in the bottom of a small cooler, place cardboard on top of the pack

3. Place the bags into the cooler, leaving room for an additional cardboard/packing layer. The bags may be covered with foam or cardboard to prevent shifting during shipment

4. Include Chief Scientist's signed original of the Chain of Custody Sheet inside each cooler [place in Ziploc bag and tape to the inside lid]. A copy of the custody sheet should be made prior to shipment

5. Samples should then be shipped via UPS Priority Overnight Service (NOTE: DO NOT ship on a Friday).

NOTE: Upon shipping samples, call or email the above contact person to provide UPS tracking air bill numbers.

Chapter 3 –Tissue Sampling For Contaminants and Histopathology

Overview

The Bioeffects Program collects tissues to assess body burdens and the effects of chemical contaminants and other stressors. The assessment of organisms for bioeffects (e.g., tumors, biomarkers, histological analysis), helps to get at the "so what " question when assessing the relationship between the presence of chemical contaminants in the environment and their impacts. This chapter addresses the collection of biota used to assess ecological effects in the Nation's coastal environment as part of the Bioeffects Program.

Collection of oysters by hand, Ayres Reef, Mesquite Bay, TX

1.0 Sampling Mollusks

The NST Bioeffect Program collects fish and mollusks including oysters, mussels, clam and conch as a part of efforts to assess the biological effects of pollutants in coastal and marine environments. Adult cockles, blue mussels, and clams are hand collected and placed in pre-labeled Ziploc bags and preserved on ice. Sufficient numbers of shellfish need to be collected to ensure adequate sample size for chemical analysis and histopathology. Depending on size, a sample should consists of a composite of 20 or more organisms. At each location, three composite samples are collected one for each analytical method (trace elements, organics and histopathology). Summaries of sample collection procedures for mollusks are described here.

1.1 Field Equipment and Materials for Collecting Mollusks for Contaminant Analyses and Histological Examination

- Electronic equipment: Handheld GPS (set to NAD83 a NPAA Chart standard), Water Quality Meter (DO, Salinity, DO Temperature), Digital Camera.
- Sampling supplies: clam rake, epibenthic dredge, Kevlar gloves, nitrile gloves (unpowdered), heavy duty Ziploc bags (1 gallon), oyster knife, snorkeling gears, cleaning gear, ice cubs, notebooks, datasheets, pens, sharpies, 48 qt cooler with drain (to store samples on ice in the field).

- Shipping supplies: strapping tapes, 28 qt coolers (no drain, for shipping chemistry samples), 16 qt coolers (no drain, for shipping histology samples), COC forms, shipping labels, ice cubs, blue ice gel,

1.2. Methods for Field Sampling of Mollusks

Oysters, cockles, mussels, and clams dwell mostly in intertidal or shallow habitats. Conch are found in the subtidal zone. Therefore, depending on the habitat, specimen sampling may be done using different methods. In general samples are hand collected by diving (SCUBA or freediving), dredging or tonging in the subtidal areas, while intertidal specimens are collected by wading or using shovels and rakes in the case of cockles and clams, which burrow in the intertidal zone.

Sorting of oysters, Biloxi, MS

Adult or edible size mollusks should be collected at low tide following the standard quality control and quality assurance protocols of the NS&T Mussel Watch Program protocol (Apeti et al., 2011; Lauenstein and Cantillo, 1998):

1. Wear personal protective gear (gloves, PFD, survival suit, as appropriate) and handle specimens with care as some bivalves have sharp edges. The Program recommends the use of Kevlar gloves.
2. Place specimens into a clean container, carefully break apart any clumps and remove adhering mud and debris.
3. Quickly sort the animals by selecting the largest size range of sufficient quantity. Only adult or edible size organisms should be selected.
4. Label two Ziploc freezer bags per station and per laboratory. Labels should include site acronym, date, and station number; double bag each sample
5. Place the animals into double labeled Ziploc freezer bags and place the bags in the ice chest for preservation.
6. Specimens should be identified in the field by common name at least .
7. At each location, it is recommended to collect about 20-30 specimens for each analytical method (trace elements, organics and histopathology). It is also recommended to err on the side of collecting more sample than less because a portion of the sample is also kept for possible retrospective analysis in the National Status & Trends contract laboratory specimen bank.

1.2.1. Precautions

- Avoid contamination of samples from oil, fuel, exhaust fumes, flaking or rusty metal or other potential forms of chemical contamination. When using a boat for sample collection, make sure that the bilge pump is off when on station.
- Avoid collection of samples on anything other than natural substrates. Untreated concrete and natural rock used for breakwaters are acceptable.
- The specimens' shells should be thoroughly rinsed in water at the site to remove mud and debris which could become sources of contamination for the tissues inside.
- Sample bags should be properly labeled to indicate site code, collection date, and station number (when appropriate).
- Put samples on water ice immediately (even pending collection of a complete sample) and maintain the sample on water ice, frequently draining the melt water. Bivalves are capable of surviving for several days if water ice is maintained and melt water is not allowed to accumulate.

1.2.2. Ancillary Measurements

Measurements of water quality (salinity, depth and water temperature), and height above the water level where samples are collected are to be taken at each site. For additional information on making water quality measurements, please refer to Chapter 2.

1.2.3. Sample Holding

DO NOT allow bivalves to freeze. Use extra caution when ambient air temperatures drop below freezing; ensure iced samples do not get too cold. This is especially important for samples that will be analyzed for histopathology/gonadal index.

Try to ship samples within 48 hrs of collection. However, experience has shown that it is better to hold bivalve samples as described above over weekends or holidays rather than risk shipping on a Friday with a Monday morning delivery.

1.3. Methods for Packing and Shipping of Mollusk Samples

"Sample shipping conditions" are NOT the same as "sample holding conditions." The water ice MUST be protected from leaking out of the cooler. An alert courier is likely to hold your shipment for perceived safety/security reasons if they observe liquid leaking from the cooler. The shipper – YOU – may be contacted by the courier and TOLD to come retrieve your "hazardous" shipment. To ensure water does not leak from the cooler double bag the water ice in Ziploc bags (Figure 1). If the shipping cooler has a drain port tape it shut.

1.3.1 Packing Samples

Samples going to separate laboratories generally should be packed in separate coolers. This should be determined before field activities begin. For example, bivalves for chemical analysis are not usually packed in the same cooler as bivalves for histopathology analysis. It is best to ship multiple small coolers (48 qt size or smaller) than one large cooler. They are easier to handle by one person. Completely fill the entire volume of the cooler with equal volumes of sample and water ice. This will help minimize sample movement. Water ice should account for at least one-third of the cooler's volume (about 10 lbs of ice for a 28 qt cooler). While gel packs may be used, water ice has proven to be more consistent. If gel packs are used they should fill at least ½ the cooler volume.

Use the Chain-of-Custody form to inventory the samples as they are placed into a shipping cooler. This process is easier and faster when done by two people; one recording and the other calling out the sample information as it is placed into the cooler.

1.3.2. Packing Precautions

1. Do NOT put ice in the bags containing the bivalves. Bivalves and water ice should be in separate bags and double bagged.

2. Do NOT allow samples to freeze (this is a caution peculiar to the Alaska and few East coast sites almost exclusively, in winter).

Bivalves can be kept alive for days when appropriately held on ice (5°C) with sample bags properly sealed and free of freshwater contact. However, proper holding and shipping within 48 hours of collection will ensure organisms arrive

alive. Periodically examine the bags of bivalves to ensure that any entrained water has not leaked into the bags. Any standing water in the Ziploc bags should be drained immediately.

1.3.3 Packing and Shipping

On the day of shipping – preferably immediately before dropping off the coolers with the courier, prepare the shipping water ice. Double bag water ice and place these bags in an empty cooler until ready to use. Select a cooler for shipping and line it with a trash bag. Place one layer of bagged water ice on the bottom in the trash bag liner. Next place the samples to be shipped into the cooler noting the sample site, and collection date on the chain of custody sheets (Figure 1). Cover the samples with at least two layers of bagged water ice.

1. Site name, collection date and station number (if appropriate) must be clearly visible (readable) if they are not you may write this information on a piece of paper and place the paper between the inner and outer double bagged samples.
2. Completely fill the cooler – fill empty space with extra bagged water ice.
3. Before taping the cooler shut, place a copy of the chain of custody form in the top of the cooler protected from moisture inside a Ziploc bag
4. Use nylon reinforced strapping tape to securely fasten the cooler lids shut (Figure 1). Use at least two bands of (3 wraps each)
5. Attach the courier's air bill to the top of the cooler. Include phone numbers of the field crew (shipper) and the lab (receiver).
6. Deliver shipping coolers to an overnight courier, an authorized agent, or call for a pickup from a secure location. Do not leave the shipping coolers at a drop box.

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Figure 1. Example of packaging of bivalves for shipping.

2.0 Collection of Fish Tissues for the National Status and Trends, Bioeffects Program: Fish Sampling and Necropsy Procedure for Contaminant Analyses and Histopathology

2. Overview

Assembly dissection of salmon in Alaska.

This section describes the standard necropsy procedures for the collection of fish tissues for chemical and histopathological analyses for the NOAA National Status and Trends, Bioeffects Program. Methods commonly used by NS&T for fish collection are also introduced. Adapted from methods used by the NOAA/National Marine Fisheries Service/Northwest Fisheries Science Center for performing fish necropsies, this standard procedure ensures collection of high quality samples resulting in accurate chemical analyses and histopathological diagnoses. In general, a number of necropsy and sample processing techniques are employed to prevent degradation or contamination of samples. After collecting the target fish species using methods such as gill nets, traps, otter trawl, etc., fish are sorted, examined, measured and dissected to remove the appropriate tissues (gill, muscle, kidney liver and stomach content). Dissections are conducted immediately after sacrificing each fish to prevent degradation of tissue due to cell death. Separate sets of dissection tools are used for external and internal procedures to prevent transfer of chemicals from the skin of the specimens to the internal tissues, or between tissues collected for analyses. Tissues for chemical analyses are kept frozen, while histopathology samples are immediately preserved in fixative to prevent tissue degradation that could interfere with accurate analyses. Proper necropsy techniques are necessary to ensure the collection of high quality samples.

2.1. Materials

Materials used for fish sampling and fish tissue collection include:

Collection Equipment may include;

otter trawl, gill nets, fishing hook/line, beam trawl, traps

Dissection equipment

Stainless steel scissors, titanium knives, Teflon® knives, polyamide forceps, scalpel

Sample management equipment

250 mL I-Chem jars, 50 mL amber vials, Cryovials, 50-ml vials with Teflon®®-lined lids, large mouth 2L plastic containers, balance, calculator, gloves, measuring board, tape measure, Teflon® cutting surfaces, tissue cassettes, large holding container

Reagents

Distilled water rinse, ethanol 70% , isopropanol rinse, Dietrich's fixative, formalin

2.2. Fish Sampling

Depending on the habitat of the monitoring site, water depth, and the target fish species, different collection methods are used. Pelagic fish are primarily collected with gill nets, while otter trawls are used mainly for ground fish. Alternatively, fish can be collected with hook and line, and occasionally, specimens from commercial landings have been collected. These alternate collection methods are necessary because larger fish, such as older Atlantic croaker, are able to avoid an Otter trawl, or are found in un-trawlable habitats such as shallow water, along marsh edges, and over oyster reefs. After collection, fishes are culled by species and measured and then enough fish are accumulated in proper age range (and sex if possible) for sampling. Fish samples are taken into a clean room and processed for necropsy, which should occur as soon after death as possible. Sample handling and preparation follow established protocols by the Northwest Fisheries Science Center (NWFSC) a division of the NOAA National Marine Fisheries Service (NMFS, 1995). The NOAA National Status and Trends, Benthic Surveillance Program also provides additional information specific to ground fish sampling and onboard handling (Lauenstein and Cantillo, 1993).

2.3. Fish Handling and Preparation

After collection, fish are sorted. Appropriate size ranges for each species are placed into a live holding container with fresh seawater. The minimum fish length is determined by the size of the animal when a given species reaches sexual maturity, if spawning condition is a consideration. To minimize stress during holding, fish are maintained either in flowing or frequently replenished seawater (Lauenstein and Cantillo, 1993). Dead animals or animals severely damaged during the capture process are culled. Each animal selected for necropsy is weighed, measured, and assigned a unique specimen number. Additional information that should be recorded on the data sheets include length (total fork length), weight, scientific name, common name if available, site number, date, collectors, environmental conditions, type, and approximate number of visible parasites, location and description of grossly visible anomalies and lesions, identity of person performing necropsy.

2.4. Necropsy

To prevent cross contamination, four sets of sampling tools are used to remove fish tissues for various analyses. One set is used to cut through the body wall or to make the initial cuts through the epidermis for fish muscle dissection for chemical analysis. A second set is used to collect the liver and other internal tissues to be analyzed for organic contaminants. The third set, consisting of a Teflon® knife and polyamide forceps, are used for collecting liver tissue for trace element analyses. The fourth set of dissection tools are used strictly for the collection of tissues for histopathological analysis. Necropsy must be performed, whenever possible at a clean work station. Tissues are collected from each specimen. Composite samples of tissue from 3 to 5 fish may be necessary to gather enough mass for chemical analyses.

To prevent transfer of contaminant residues, all necropsy tools and the Teflon® cutting surfaces are cleaned prior to use and before the next fish as follows:

- Between individual fish of one species at the same site, the tools and the Teflon® cutting surfaces are wiped and rinsed with distilled water.
- Between sites, and between different species within sites, the tools are washed and rinsed using a thorough soap and water wash, followed by a distilled water rinse; an isopropanol rinse; another distilled water rinse; and air drying.

2.4.1 Observation and Collection of External Lesions

Any grossly visible external lesions on each animal is described and recorded on the field card. Using the set of external tools, tissue specimens from grossly visible external lesions on the fin and skin are excised to a 3-mm thickness, and placed into tissue cassettes and preserved in Dietrich's fixative (Gray, 1954) at room temperature.

2.4.2. Collection of Otoliths for Fish Age Determination

Fish are sacrificed by severing the spinal cord just posterior to the brain; and otoliths, scales or spines are collected. Otoliths are collected by opening the fish's cranial cavity to expose the brain. Using a titanium knife, a deep vertical cut is made from the top of head in line of the base of the operculum followed by a horizontal cut just above the eyes which allows the removal of the cranial bone and the exposure of the cranial cavity. Forceps are used to take the otoliths. Otoliths from flatfish are stored dry in a test tube labeled with the specimen number, and otoliths from round fish are stored in 70% ethanol (Lauenstein and Cantillo, 1993). Scales or spines may be collected from species that had otoliths that are difficult to assess for age, or where these structures provide a more accurate assessment of fish age. Collection of other fish tissues begins immediately after collection of structures for aging.

2.4.3. Collection of Gill Tissue

Gill tissue is collected using surgical scissors after removing the operculum. The tools used for external tissue collections are used. A pair of scissors is used to remove the operculum and expose the gills. Another pair of scissors is used to cut the interior gill arches which are placed on the Teflon® board. A clean scalpel is then used to cut and isolate gill filaments, which are individually placed in cassettes and preserved in 2-L container containing Dietrich's fixative.

2.4.4. Collection of Bile

The body cavity is opened using the external tools. Any grossly visible anomalies and lesions in the internal organs are described and recorded on the field card.

A separate set of isopropanol-rinsed dissection instruments are used to collect target internal tissues. The entire liver is excised, with gall bladder attached, and placed on an isopropanol rinsed Teflon® board. The gall bladder is then carefully separated from the liver, gripping the bile duct to prevent bile from flowing out of the bladder, and care is exercised not to touch the liver with the scissors. Care is taken not to spill any bile onto the liver, otherwise the tissue will be contaminated with bile fluid and deteriorate due to direct contact with the bile. If bile does inadvertently contact the liver, the affected portion of the liver is removed and discarded immediately. The bladder is held over the mouth of the amber vial designated for bile collection. A clean #11 scalpel blade is used to puncture the bladder and the bile is allowed to drip down the tip of the blade into the vial.

2.4.5. Collection of Liver for Histology

Since tissue degradation of cellular structures begins immediately after death of the fish, all histology samples are collected as soon as possible after sacrifice. Liver tissue for metals analyses are collected before the histopathology sample is removed. This prevents the metal "internal" set of dissecting tools from touching the liver before the metal sample is collected.

A pair of sharp scissors from the set of internal tools is used to cut a routine section for histology (no thicker than 3 mm) from the central longitudinal axis of the liver (Figure 2). The cut side of the liver tissue is placed face down into

a cassette, with care taken to avoid mechanical damage such as tearing, poking, pulling, or stretching of tissues. For optimal fixation, tissues in the cassette should not contact one another or the sides of the cassette. As soon as a cassette is filled, it is immediately placed into the jar containing Dietrich's fixative.

If grossly visible lesions are observed in the liver, sections for histology are also taken for as many of the differing lesion types as possible. Whenever grossly visible nodules are noted in the liver, sections no thicker than 3 mm are also collected from the heart, upper intestine and spleen for histology. These tissue samples are used to verify presence/absence of metastatic foci from primary neoplasms in the liver.

2.4.5.1. Handling Histopathology Samples

All histopathology tissue samples are preserved in a 2-L container with Dietrich's fixative. To achieve optimal fixation, the volume of fixative to tissue should be 1:20, therefore no more than 30 cassettes containing the tissues are placed into a container. Tissues are allowed to remain in this fixative for at least 48 hr and no more than five days. Fixation at normal room temperature is optimal. However, if fixation at 4-5°C is necessary, then a minimum of 72hr is required for complete fixation. Periodic agitation of containers is important during the first 48 hr to assist uniform penetration of the fixative. Once fixation is complete, cassettes are transferred to 70% ethanol for storage until processed in the laboratory.

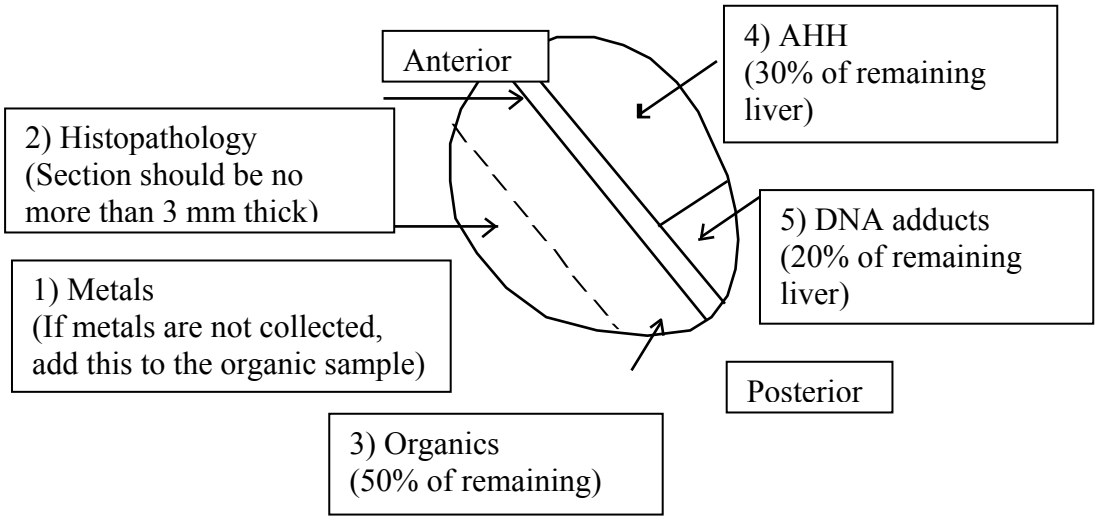


Figure 2. Generic fish liver showing where various samples were collected, and the order in which they were collected. Percentages show approximate amount of liver needed after collection of the histopathology and metal samples.

2.4.5.2. Collection of Liver Tissue for Metals Analyses

Liver tissue collection for metal analysis is quickly performed just after bile collection such that the liver is not contaminated with metal dissecting tools. Polyamide forceps and a titanium knife rinsed with isopropanol followed by deionized water are used. The polyamide forceps are used to place the tissue into the acid-rinsed 250 mL I-Chem jars. Samples are kept frozen in a freezer or on dry ice. Liver samples for metal analyses are generally from the same area of the liver for each fish, as shown in Figure 2. Composite sample from 3-5 fish may be necessary to gather enough tissue for analysis

2.4.5.3. Collection of Liver Tissues for Organic Contaminants Analyses

After samples for histology and trace metal analysis are collected, the remaining liver is divided into proportions for the following analyses: 50% of the liver for organic chemical analyses, 30% for aryl hydrocarbon hydroxylase (AHH), and 20% for xenobiotic-DNA adducts. Liver samples for organic chemistry analyses are also routinely collected from the same area of the liver for each fish, as shown in Figure 2. The minimum amount of tissue needed for each analysis is: for organics, 1 g; for AHH, 500 mg; and for DNA adducts, 200 mg. If an individual liver was too small to provide this minimum amount of liver for each sample, then these samples may be composited back in the lab. If the size of individual samples is so small that they would have been freezer burned, compositing needs to take place in the field.

AHH and DNA adduct samples are placed into cryovials labeled with the unique specimen number and immediately frozen in liquid nitrogen. These samples are later transferred to -80°C freezers for storage. Tissues collected for organic chemical analyses are placed into similarly labeled dichloromethane-rinsed scintillation vials with Teflon®-lined lids. These samples could be kept on ice at the necropsy station for up to 15 min until they are placed into a -20°C freezer for storage. These samples are stored in a -80°C freezer after they had been transferred to the lab.

2.4.6. Collection of Kidney and Ovary Tissues for Histology

After the histology liver samples had been collected, additional tissues such as kidney and gonad could be collected for histology. The internal tools are used to collect sections less than 3-mm thick for these organs. The kidney histology sample is collected as a sagittal or transverse section from the posterior kidney depending on the species, and the gonad sample as a transverse section from the middle of the ovary or testis. Additional cassettes are used if the cassette containing the liver is full. The kidney and gonad sections are placed flat in the cassette, and then placed in fixative.

2.4.7. Collection of Stomach Contents

Contents of the stomach (the portion between the esophagus and the pyloric ceca) are removed by grasping one end of the stomach with internal dissecting tools and gently squeezing the contents into appropriate containers. Stomach contents for organic analyses are placed into dichloromethane-rinsed, 50-ml vials with Teflon®-lined lids, and held on ice for a few minutes until they are placed into a -20°C freezer, followed by transfer to a -80°C freezer at the laboratory. Stomach contents collected for taxonomy are composited with 5 individuals of the same species in one 4 oz glass jar, and preserved with neutral buffered Formalin.

2.4.8. Collection of Fish Muscle Tissue

Fish muscle tissue is removed by placing the eyed or left side facing up. Using a scalpel series of three cuts were made to expose a rectangular subsection of muscle behind the head and between the lateral line and dorsal fin. The scalpel is wiped and rinsed with distilled water between cuts to remove scales and mucus. The first cut is 100 to 150 mm long (depending on the size of the fish) and extends from behind the head parallel to and about 5 to 10 mm dorsal to the lateral line. The next cut is above and parallel to the first, just below the fin ridge. Then the third perpendicular cuts are made along the lateral line.

A scalpel is used to lift the edge of the skin along the cut line at the end of the rectangular cut. The fish is held with one hand and the edge of the skin is pulled back using forceps or a hemostat held in the other hand. The skin is pulled back from the rectangular cut to expose the muscle tissue mass.

A layer of adipose tissue lies along the dorsal fin ridge. This tissue is not to be taken with the muscle tissue subsample because it is fattier than the other muscle tissue and may contain more organic contaminants than the rest of the muscle tissues.

Removal of skin using 'exterior' tools.

Another set of Teflon®/titanium knives is used to take the "core" of the muscle tissue mass within the rectangular cut that was cut free and removed with a titanium knife. Extreme care is taken to assure that neither the contaminated rectangular cut line nor the fish exterior is contacted either by the titanium knife or by the cored muscle sample. Polyamide forceps are used to transfer muscle tissues to 250 mL I-Chem containers for samples to be analyzed for chemical contaminants (metals and organic). Contaminants are found in lower concentrations in fish muscle tissues than in other tissues, such as liver, therefore prevention of sample contamination is extremely important.

Removal of muscle using 'interiorr' tools.

2. 5. Methods for Packing and Shipping of Fish Tissue Samples

2.5.1. Packing Samples

Samples going to separate laboratories should be packed into separate coolers. It is best to ship multiple small coolers (e.g. 48 qt size or smaller) than one large cooler. Bubble bags are used to protect glass jars, and coolers are completely filled with equal volumes of sample, water ice/gel pack ice or dry ice based on the sample preservation method.

Chain-of-Custody forms to inventory the samples are placed in each shipping cooler as they are filled out. To prevent the COC from being damaged by moisture, the forms are inserted inside double Ziploc bags before they are placed in the coolers.

2.5.2 Shipping

Each cooler is tightly closed using Nylon reinforced strapping tapes to securely fasten the cooler lids shut. The courier air bill is attached to the top of the cooler. Include phone numbers of the field crew (shipper) and the lab (receiver).

2.5.3. Precaution

Because shipping samples preserved with chemical reagents or on dry ice are considered hazardous materials, special hazmat forms are filled out based on regulation. Deliver shipping coolers to an overnight courier, an authorized agent, or call for a pickup from a secure location. Do not leave the shipping coolers at a drop box.

Figure 3. Typical external fish anatomy.

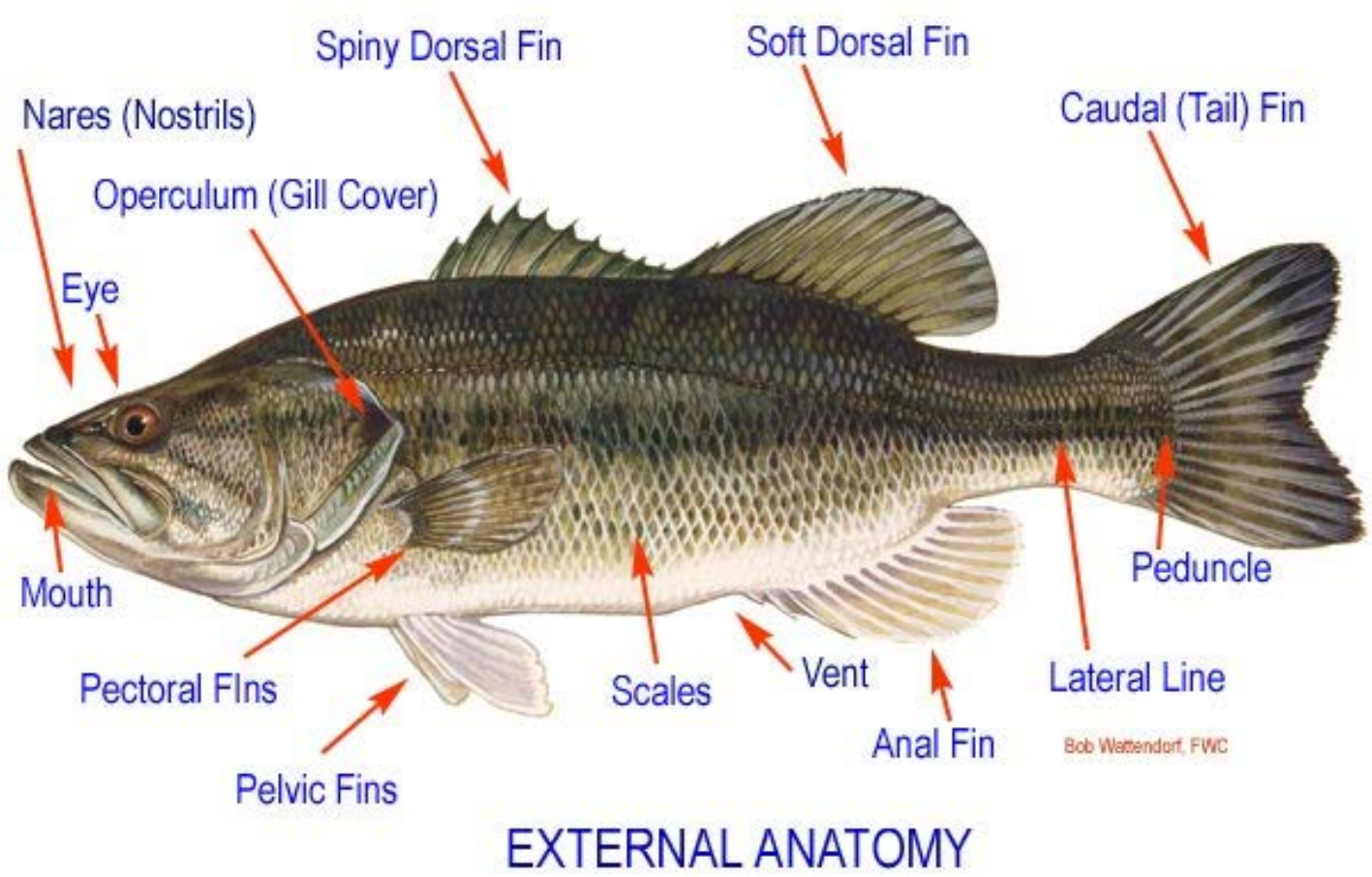
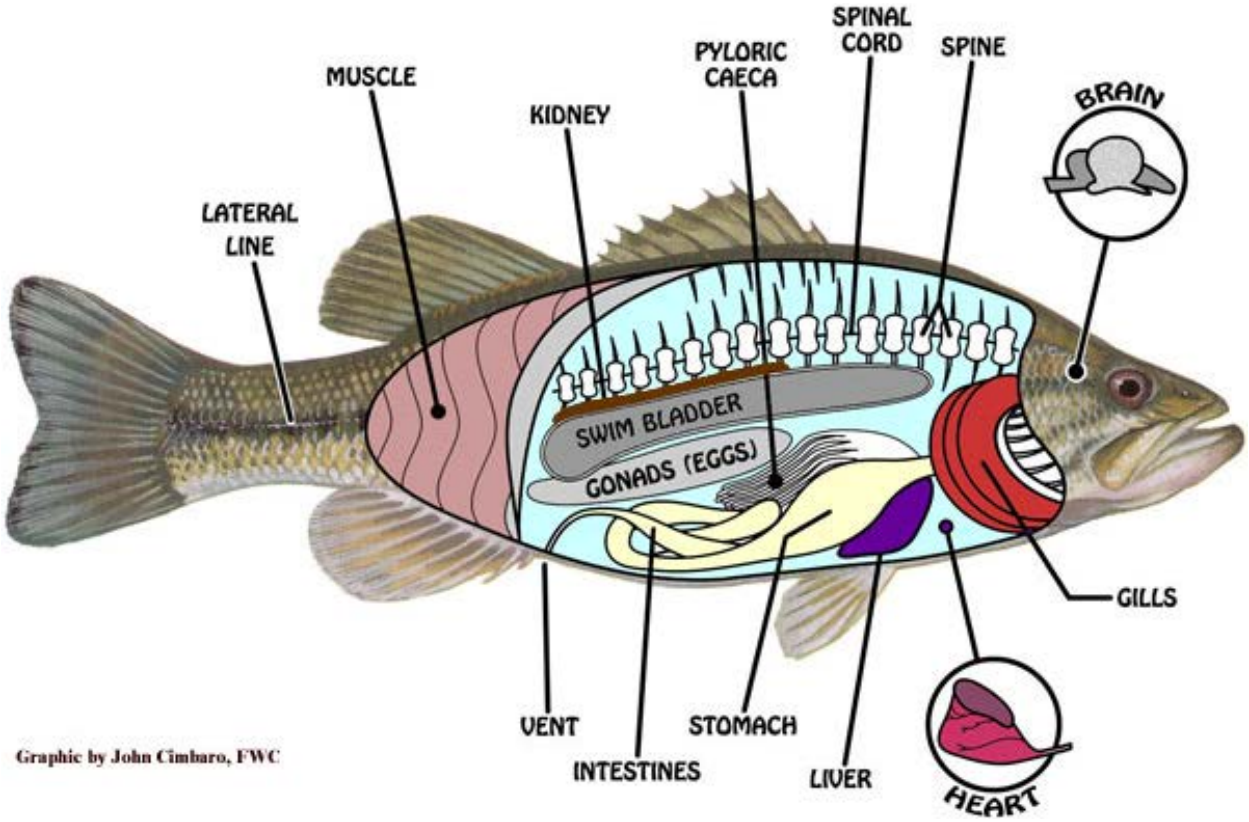


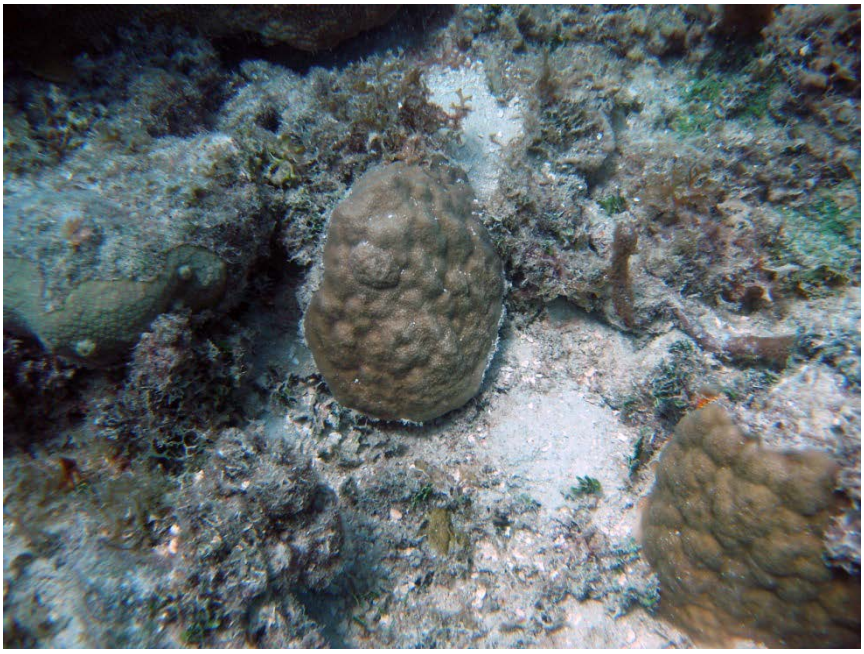
Figure 4. Typical internal fish anatomy.



3.0. Sampling Corals

Overview

The assessment of corals is a relatively new activity in the NS&T Program. Corals were sampled and analyzed beginning in 2005. The coral that has been analyzed as part of the NS&T Program is mustard hill coral, or Porites astreoides. This species was chosen as it is common in Florida, the Bahamas and the Caribbean (Humann and DeLoach, 2002). Colonies of P. astreoides are generally massive but can also be found as encrusting forms, particularly in shallow, surging waters (Veron, 2000). Collecting permits are usually easier to obtain for this species, as it is not endangered. The coral can be collected by SCUBA diver with a hammer and coral punch. Coral samples are analyzed for chemical contaminants, and histological condition.



Encrusting form of Porites astreoides, similar to the size of the colonies sampled. Diameter of colony shown is approximately 25 cm.

CCMA diver sampling the coral P. astreoides in Vieques, Puerto Rico.

3.1. Sampling Corals for Chemical Contaminant Analysis

3.1.1. Field Equipment and Materials for Collection of Corals for Chemical Contaminant Analysis – Hammer, titanium punch, sediment jars

3.1.2. Methods – NOAA maintains a rigorous diving safety program and all SCUBA operations are coordinated with the NOAA dive master and executed by certified NOAA divers. Coral samples for chemical contaminant analysis are taken using a hammer and titanium punch. Titanium is used as it is virtually inert and is not a target trace element for NS&T. Prior to each use, the punch is rinsed with acetone to minimize cross-contamination between sites. Divers collecting the coral samples wear disposable nitrile gloves to avoid contaminating the coral samples. The diver hammers the titanium punch into the coral head which produces a coral core with a diameter of approximately 1.5 cm and a similar core length. Approximately 20 cores are taken at each site and placed in an IChem® certified clean 250 ml jar and then capped underwater. The jar is brought to the surface, drained of water and placed on ice. At the end of each day, the samples are placed in a freezer (-15 ºC), and at the end of the mission, samples are shipped overnight to the laboratory for analysis. A series of water parameters (dissolved oxygen, temperature, salinity, and conductivity) are also measured at the surface and bottom at each site using a YSI® salinity/conductivity/temperature meter or similar equipment.

3.2. Sampling Corals for Histological Examination

3.2.1. Field Equipment and Materials for Collection of Corals for Histological Examination – Hammer, 2.2 cm ID stainless steel punch, 50ml Falcon® centrifuge tubes, Z-Fix solution.

3.2.2. Methods

Field samples are collected using larger stainless steel coring tubes (2.2-cm inside diameter) to provide sufficient undamaged tissue away from the edges of the core. One or two core samples are taken from up to five selected coral colonies at each stratum, depending on success in locating sufficient coral colonies in each sampling stratum. Samples will be stored in 50-mL Falcon™ tubes filled with seawater and Z-Fix concentrate diluted with seawater (1 part concentrate and 4 parts seawater). Preserved samples are shipped to the analytical laboratory. Samples may be shipped en masse, or in smaller batches, depending on field logistics

3.3. Methods for Packing and Shipping Coral Samples

Coral samples for histological analyses do not need to be frozen or refrigerated as they are immediately placed in a preservative Z-Fix solution. The sample tube caps should be sealed individually with parafilm or vinyl tape prior to shipping. Shipping occurs at the end of the sampling cruise.

Before transport:

1. Check each container lid to assure that it is taped with the labeling clearly legible on the lids and sides of the tubes. Verify the lids and labels match.

2. Place tubes in trays if available and then place directly into a Ziploc bag to prevent leakage of the tubes and seal the bag.

3. Put vermiculite in the bottom of a cooler.

4. Place the “bagged” tubes into the cooler, leaving room for additional vermiculite. Once all samples are stowed, fill all empty spaces with vermiculite to protect against spills or leakage.

5. Include Chief Scientist’s signed original of the Chain of Custody Sheet inside each cooler [place in Ziploc bag and tape to the inside lid]. A copy of the custody sheet should be made prior to shipment

6. Samples should then be shipped via UPS Standard Service.

3.3. Sampling Corals for Genomic Analysis

3.3.1. Field Equipment and Materials for Collection of Corals for Chemical Contaminant Analysis – Hammer, 2.2 cm ID stainless steel punch, 50ml Falcon® centrifuge tubes, TRIzol* solution.

* TRIzol is extremely caustic and carcinogenic. Always wear gloves when pouring or handling tubes. Wash skin thoroughly with water if a spill occurs. For more information on TRIzol, please see the manufacturer’s website:
http://products.invitrogen.com/ivgn/product/15596026
MSDS: https://tools.invitrogen.com/content/sfs/msds/2012/15596026_MTRNALT_EN.pdf

3.3.2. Methods – Coral samples for genomic analysis are taken using a hammer and large stainless steel coring tubes (2.2-cm inside diameter). Cores are taken at each site and placed in an IChem® certified clean 250 ml jar and then capped underwater. The jar is brought to the surface where surface personnel wearing nitrile gloves remove the core from the stainless steel tube and place the sample in pre-labeled sample tubes containing 10-15mL of TRIzol. Each sample tube should then be sealed with parafilm or vinyl tape to prevent leakage and placed in a holding tray. At the end of the mission, samples are shipped to the laboratory for analysis. A series of water parameters (dissolved oxygen, temperature, salinity, and conductivity) are also measured at the surface and bottom at each site using a YSI® salinity/ conductivity/temperature meter or similar equipment.

3.2.3. Methods for Packing and Shipping Coral Genomic Samples

Coral samples for genomic analyses do not need to be frozen or refrigerated as they are immediately placed in a preservative TRIzol* solution. The sample tube caps should be sealed individually with parafilm or vinyl tape prior to shipping. Shipping occurs at the end of the sampling cruise.

Before transport:
1. Check each container lid to assure that it is taped with the labeling clearly legible on the lids and sides of the tubes. Verify the lids and labels match.

2. Place tubes in trays if available and then place directly into a Ziploc bag to prevent leakage of the tubes and seal the bag.

3. Put vermiculite in the bottom of a cooler.

4. Place the “bagged” tubes into the cooler, leaving room for additional vermiculite. Once all samples are stowed, fill all empty spaces with vermiculite to protect against spills or leakage.

5. Include Chief Scientist’s signed original of the Chain of Custody Sheet inside each cooler [place in Ziploc bag and tape to the inside lid]. A copy of the custody sheet should be made prior to shipment

6. Samples should then be shipped via UPS Standard Service.

Chapter 4: Sampling Water For Nutrients and Turbidity/Suspended Solids

Overview

Basic water quality data includes water clarity (turbidity), temperature, salinity and dissolved oxygen. These parameters have been addressed in the previous chapters. Nutrients and suspended sediment are two additional measures of water quality that impact both benthic and pelagic communities. Eutrophication is arguably a more pervasive problem in coastal waters than toxic contaminants. Nutrient loads and suspended solids are particularly important in coral reef

systems due to the sensitivity of many coral species. Nutrient release is also becoming an important issue at higher latitudes as global climate change has the likelihood of releasing vast stores of nutrients now stored in permafrost.

1.0 Nutrient Sampling

Excess nutrient fluxes to coastal ecosystems can have cascade of environmental effects including shifts in algal community composition, harmful algal blooms, hypoxia, decreases in water clarity and negative effects on coral reefs. Water column nutrient sampling can provide insight into the magnitude and dynamics of potential nutrient pollution issues in a system. Because nutrient concentrations change relatively rapidly over time (e.g. seasonally and/or in response to rainfall/runoff events), recurring sampling (e.g. weekly or monthly) is recommended.

1.1 Field Equipment and Materials

Logistics: GPS, roadmaps and nautical charts, transportation (boat, automobile), target sampling list.

Sampling equipment: Sample bottles (HDPE), sharpies, data sheets on waterproof paper, ice, pens, digital camera , gloves. YSI or Hydrolab multimeter, secchi disk or turbidity meter, Nisken bottle/CTD/Rosette sampler.

1.2 Nutrient Sampling Methods

Nutrient bottles should be high density polyethelene (HDPE) and pre-cleaned prior to entering the field. Field technicians should confirm with the principal investigator as to how much sample is required, but generally 100 ml is sufficient.

As stated in chapter 2, nutrient samples should be collected prior to any benthic sampling to avoid contaminating the water sample with sediment stirred up during benthic sampling. Field personnel should wear disposable nitrile gloves to avoid contaminating the sample. Other sources of nutrient contamination include boat exhaust, cigarette smoke and human breath. Proper steps should be taken to avoid exposing the sample to these sources (e.g.no smoking, sample with boat engine off, keep bottles capped except when sampling).When arriving on site, the bottle should be rinsed three times with site water. For surface water samples, the bottle should be submerged at a depth of 0.5m. At least 10% headspace should be left to allow for expansion during freezing.

If bottom water samples are desired, a Niskin bottle should be deployed to sample bottom water. The bottle should be cleaned (HCl and DI water) prior to sampling and in between sites. For samples at depth, sample bottles should be rinsed 3 times with water from depth (rather than surface water).

Bottles should be labeled with indelible marker with the site name, date and depth (surface, bottom or depth in meters, as appropriate).

1.3 Sample Handling and Shipping

Samples should be stored on ice in the field. If the samples are to be filtered prior to analysis, filtration should occur the same day as sampling. Water should be filtered through Whatman GF/F (0.7 micron) filters at a vacuum of no more than 7 inches of Hg. Glassware and filter apparatus must be cleaned with HCl (10%) and nanopure water (3 rinses) prior to filtration and between each sample to remove the possibility of cross sample contamination.

Samples should be stored in a freezer. Prior to shipping, it is desirable to completely freeze the samples (e.g. overnight). Coolers should be packed with blue ice or water ice with any extra space padded with newspaper or foam (so samples do not bounce around during shipping). Samples should be shipped overnight to the laboratory.

2.0 Total Suspended Solids

Suspended materials in the water column can negatively impact ecosystems in a variety of ways, including reducing photosynthetically active radiation available for seagrasses and other benthic algae, changing the depth of the photic zone and eventually settling out of the water column and affecting benthic fauna. While a turbidity meter can give a reasonable estimate of water clarity, it may be more useful to obtain total suspended solids information.

2.1 Field Equipment and Materials

Logistics: GPS, roadmaps and nautical charts, transportation (boat, automobile), target sampling list.

Sampling equipment: Sample bottles (HDPE), sharpies, data sheets on waterproof paper, ice, pens, digital camera , gloves. YSI or Hydrolab multimeter, secchi disk or turbidity meter, Nisken bottle/CTD/Rosette sampler.

2.2 TSS Sampling Methods

Plastic sample bottles are recommended in order to avoid breakage. At each site, one liter water sample is collected by submerging a clean, labeled bottle approximately one half meter below the water surface. The bottle should be labeled with the site name, date and depth (if applicable). The bottle will then be capped and placed on ice in a cooler.

2.3 Sample Handling and Shipping

Samples should be stored on ice or refrigerated . If samples need to be shipped to a laboratory, overnight shipping is preferred (on blue or water ice).

Back at the laboratory, the one liter sample will be filtered through a dried, pre-weighed glass fiber filter. The exact volume of water filtered will be noted, but the entire sample should be filtered. The filtered solids will then be rinsed several times with deionized water in order to remove salts, and the filter will be dried and then weighed to calculate TSS in mg/L.

TSS (mg/L) = ([A-B]*1000)/C

Where A = End weight of the filter in grams (g)

B = Initial weight of the filter in grams (g)

C = Volume of water filtered in liters (L)

APPENDIX 1 – Important Contacts

NOAA Contacts

General inquiries should be addressed to the "Bioeffects Program Manager" at

NOAA, N/SCI1
1305 East West Highway
Silver Spring, MD 20910
Phone: 301-713-3028
Monday – Friday 7AM to 4:30PM

Field crews actively involved in sampling should follow pre-arranged instructions for 24/7 communication.

Current NS&T Bioeffect Staff Contacts:

Ian S. Hartwell, PhD.	ian.hartwell@noaa.gov
Anthony Pait, PhD.	tony.pait@noaa.gov
Dennis Apeti, PhD.	dennis.apeti@noaa.gov
Ed Johnson, PhD.	ed.johnson@noaa.gov

Laboratories (I need to have potential lab info. here)

Amphipod samples will be shipped to:
Contractor Contact
Contractor Address (street address, not PO address)
Contractor telephone
Contractor Fax

Porewater samples will be shipped to:
Contractor Contact
Contractor Address (street address, not PO address)
Contractor telephone
Contractor Fax

Sediment chemistry and grain size samples will be shipped to:
Contractor Contact: TDI-Brooks Inc. Attn: Amanda Fryer
Contractor Address: 1902 Pinon Drive, College Station, TX 77845
Contractor telephone: 979-693-3446

APPENDIX 2 – Bioeffect Sampling Supply Check List

Sampling Supplies
Batteries
Bungie cords
Clip board
Coolers W/drains
Data sheets
Eye rinse
First aid kit
Flashlights
Gloves (nitrile diposable -medium, large, Xlarge)
Gloves (leather work)
Gloves (neoprene - cold water locations)
Gloves (playtex)
Hoses with nozzle
Ice
Kimwipes
Plastic tie wraps
Powder funnel
Rubbermaid plastic tubs with lids (benthos sample storage)
Ruler
Scrub brushes
Sediment bucket - HDPE- 5 gal
Sediment bucket lid remover
Sediment bucket lid -HDPE - 5 gal
Sediment bucket liners - HDPE - 5 gal
Seive Buckets
Sharpies, pens, pencils
Spill Kit
Sponges
Sprayers (3 gal pump up)
Squeeze Bottle (distilled water)
Squeeze Bottle (acetone)
Syringe and tygon tubing
TFE coated or stainless steel scoops
Towels - paper
Towels -cloth
Tweezers
Two-way radios

APPENDIX 2 (CONTD) – Bioeffect Sampling Supply Check List

Equipment
Digital camera
Drill battery charger
Extra clasps for grab doors
GPS
Grab - Large Young modified van Veen
Grab - PONAR
Grab frame "snowshoe" large
Grab frame "snowshoe" small (dia 32")
Grab Frame large
Grab Frame small
Grab stand
Grab weights large frame
Grab weights small frame
Nisken bottle
Sediment mixer drill
YSI or hydrolab
Sample Containers
Chem- Ichem jars 250ml-organic & metals
Grain Size/chem- Whirl-Paks
Tox-Ichem jars 250ml-HRGS P450/Microtox
Tox-1L plastic-Amphipod
Tox-1 gal plastic-Sea Urchin (porewater)
Histoplex benthos jars 1000ml
Histoplex benthos jars 500ml
Histoplex benthos jars 250ml
Clear label tape
Electrical tape
Sample jar labels

APPENDIX 2 (CONTD) – Bioeffect Sampling Supply Check List

Chemicals
Acetone
DI Water
Dish soap
Formalin
HCl (5% v/v)
Rose Bengal
Borohydride
Shipping Supplies
Blue ice
Bubble bags for Ichem 250 ml jars
Chain of custody forms
Coolers W/O drains
Duct Tape
Plaste trash bags
Plastic sleeves for Airbills
Strapping tape
Tape dispensers
UPS Airbill envelopes
UPS Airbills
UPS Dry Ice shipping labels
UPS labels to contractors
UPS lables to office
Vermiculite CoPack V175
Ziploc bags

Appendix 3 - Field sampling datasheet

"YEAR" ..... "PROJECT"..... NS&T Bioeffects Assessment

Stratum	Site	Alternate	Date	Time	Depth
					Ft M

SAMPLES TAKEN							
Benthos	Depth	OrganicTOC	Metals	Grain	Other	Other	Tox
	cm						

|# grabs for chem/tox composite

STATION LOCATION

TARGET COORDINATES	MEASURED COORDINATES
Latitude	
Longitude	

SEDIMENT DESCRIPTION							
TEXTURE		COLOR		ODOR/SHEEN		BENTHOS	
Silt		Black		None		None	
Clay		Brown		Sulfur		Worms	
Mud		Gray		Sewage		Tubes	
Sticky		Green		Oily		Mollusks	
Sand		Rust		Other*		Crustacean	
Shell/Rock		Other*				Echinoderms	
Other*						SAV	
						Algae	
						Other*	
(*describe in comments)							

WATER QUALITY	
SURFACE	BOTTOM
Temperature	
Salinity	
Dissolved Oxygen	
Secchi Depth	

SAMPLE TEAM	
	Recorder

COMMENTS

APPENDIX 4 - Sample Labels

Sample jar label

NOAA <project name>
Stratum_____ Site_____ _____of_____
Date (mm/dd) _____/_____
Taxonomy - preserved
comment_____

NOAA <project name>
Stratum_____ Site_____
Date (mm/dd) _____/_____
Amphipod/Porewater Bioassay - Refrigerate
comment_____

NOAA <project name>
Stratum_____ Site_____
Date (mm/dd) _____/_____
Metals - freeze
comment_____

NOAA <project name>
Stratum_____ Site_____
Date (mm/dd) _____/_____
Grain Size - Refrigerate
comment_____

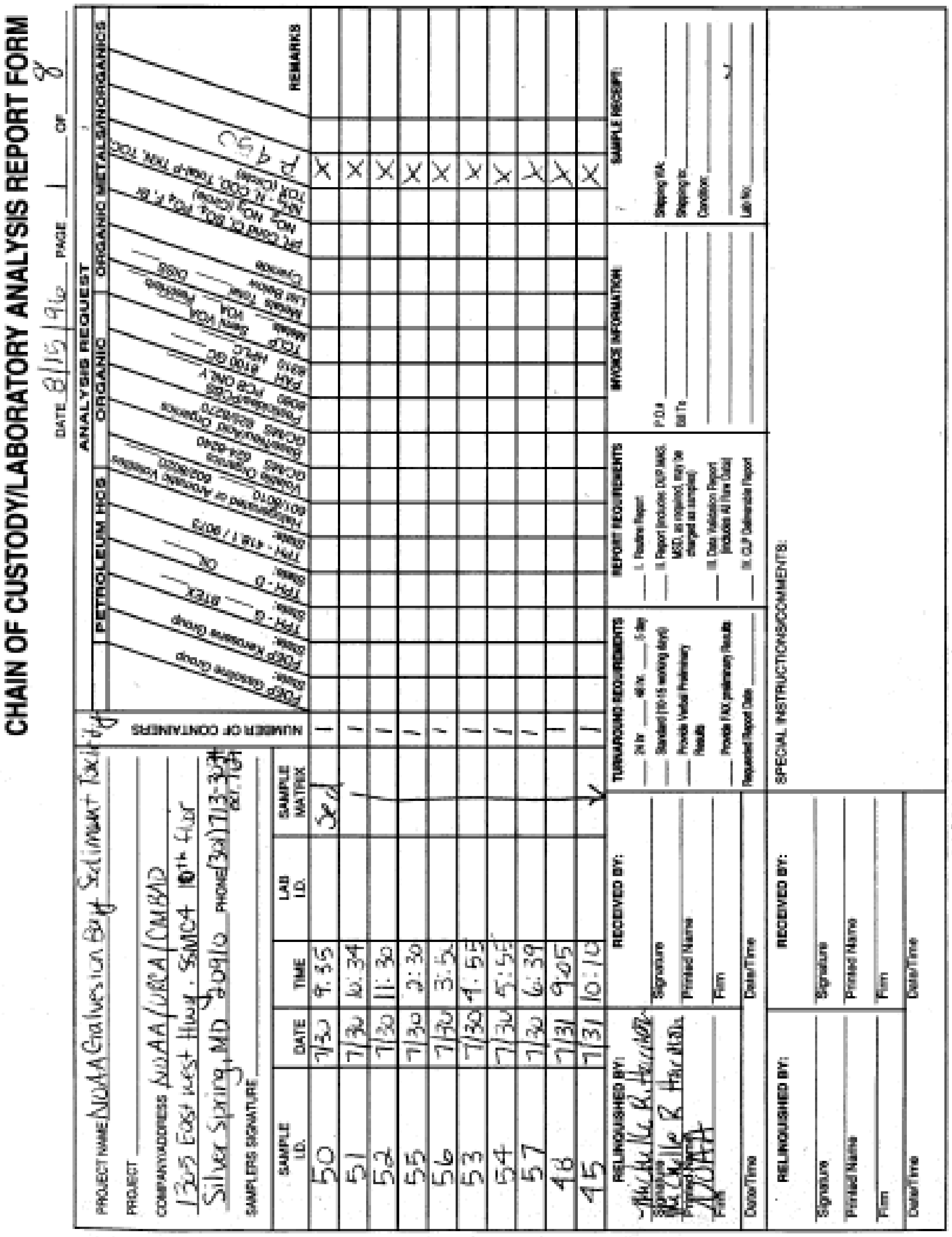
NOAA <project name>
Stratum_____ Site_____
Date (mm/dd) _____/_____
PAHs/TOC - freeze
comment_____

NOAA <project name>
Stratum_____ Site_____
Date (mm/dd) _____/_____
P450/Mtx -Bioassay freeze
comment_____

Appendix 5 - Examples of photographic documentation

6/15/2011 St Thomas East End Reserve, Station 2, grab 1

Appendix 6 - Example of chain of custody datasheet



CHAIN OF CUSTODY/LABORATORY ANALYSIS REPORT FORM

DATE 8/15/96 PAGE 1 OF 8

PROJECT NAME NOAA Galveston Bay Sediment Toxicity

PROJECT

COMPANY/ADDRESS NOAA/ORCA/CMBAD
1305 East West Hwy. SSMC4 10th floor
Silver Spring, MD 20910 PHONE (301) 713-3034 EXT. 164

SAMPLERS SIGNATURE

SAMPLE I.D.	DATE	TIME	LAB I.D.	SAMPLE MATRIX	NUMBER OF CONTAINERS	ANALYSIS REQUEST
50	7/30	9:35		Sed	1	X
51	7/30	10:34			1	X
52	7/30	11:30			1	X
55	7/30	2:30			1	X
56	7/30	3:52			1	X
53	7/30	4:55			1	X
54	7/30	5:55			1	X
57	7/30	6:39			1	X
48	7/31	9:05			1	X
45	7/31	10:10			1	X

ANALYSIS REQUEST: PETROLEUM HCS; ORGANIC; ORGANIC METALS/INORGANICS; REMARKS

RELINQUISHED BY: Michelle R. Harmon — Signature; Michelle R. Harmon — Printed Name; NOAA — Firm; Date/Time

RECEIVED BY: Signature; Printed Name; Firm; Date/Time

TURNAROUND REQUIREMENTS: 24 hr, 48 hr, 5 day; Standard (10-15 working days); Provide Verbal Preliminary Results; Provide FAX preliminary Results; Requested Report Date

REPORT REQUIREMENTS: I. Routine Report; II. Report (includes DUP, MS, MSD, as required, may be charged as samples); III. Data Validation Report (includes All Raw Data); IV. CLP Deliverable Report

INVOICE INFORMATION: P.O.#; Bill To

SAMPLE RECEIPT: Shipping VIA; Shipping to; Condition; Lab No.

RELINQUISHED BY: Signature; Printed Name; Firm; Date/Time

RECEIVED BY: Signature; Printed Name; Firm; Date/Time

SPECIAL INSTRUCTIONS/COMMENTS:

REFERENCES

Chapman, P.M., R.N. Dexter, and E.R. Long. (1987). Synoptic measures of sediment contamination, toxicity and infaunal community structure (the Sediment Quality Triad). Mar. Ecol. Prog. Ser. 37:75-96.

Heimbuch, D., H. Wilson, J. Seibel, and S. Weisberg. 1995. R-EMAP Data Analysis Approach for Estimating the Portion of Area that is Subnominal. Report prepared for US Environmental Protection Agency, Research Triangle Park, NC, 22 pp.

U.S. EPA, Environmental Monitoring and Assessment Program (EMAP). (2001.). National Coastal Assessment Quality Assurance Project Plan 2001-2004. United States Environmental protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. EPA/620/R-01/002.

National Status and Trends Bioeffects Program: Field Methods

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