Region 4
U.S. Environmental Protection Agency
Science and Ecosystem Support Division
Athens, Georgia

OPERATING PROCEDURE

Title: **Marine Macroinvertebrate Field Sampling**

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Revision History

The top row of this table shows the most recent changes to this controlled document. For previous revision history information, archived versions of this document are maintained by the SESD Document Control Coordinator on the SESD local area network (LAN).

History	Effective Date
SESDPROC-511-R4, *Marine Macroinvertebrate Field Sampling*, replaces SESDPROC-511-R3. **Signature Page:** Updated to reflect recent Divisional reorganization **General:** Corrected typographical, grammatical, and/or editorial errors. **Section 1.4:** Revised discussion to include different grab/sample sizes and adjustments for sediment consistency as well as a discussion of tissue preservative. **Section 1.5:** Included a reference to the 2015 Vittor and Assoc. White Paper comparing different macroinvertebrate sampling methods. Updated the Dive Safety Manual Reference to v1.3. **Sections 1.6 and 2.1:** Changed the reference to the use of 10% formalin as a preservative which SESD no longer uses, to the use of a non-toxic preservative such as NOTOXhisto® and added precautions such as spill containment.	September 8, 2017
SESDPROC-511-R3, *Marine Macroinvertebrate Field Sampling*, replaces SESDPROC-511-R2.	June 28, 2013
SESDPROC-511-R2, *Marine Macroinvertebrate Field Sampling*, replaces SESDPROC-511-R1.	December 7, 2009
SESDPROC-511-R1, *Marine Macroinvertebrate Field Sampling*, replaces SESDPROC-511-R0.	November 1, 2007
SESDPROC-511-R0, *Marine Macroinvertebrate Field Sampling*, Original Issue	February 05, 2007

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1 General Information

1.1 Purpose

The purpose of this procedure is to document both general and specific procedures, methods and considerations to be used and observed when collecting marine macroinvertebrate samples.

1.2 Scope/Application

This document describes specific methods to be used by field personnel when collecting marine macroinvertebrate samples. On the occasion that Science and Ecosystem Support Division (SESD) field personnel determine that any of the procedures described in this section are inappropriate, inadequate or impractical and that another procedure must be used to obtain a marine macroinvertebrate sample, the variant procedure will be documented in the field log book, in accordance with the SESD Operating Procedure for Logbooks (SESDPROC-010), along with a description of the circumstances requiring its use. Mention of trade names or commercial products in this operating procedure does not constitute endorsement or recommendation for use.

1.3 Documentation/Verification

This procedure was prepared by persons deemed technically competent by SESD management, based on their knowledge, skills and abilities and has been tested in practice and reviewed in print by a subject matter expert. The official copy of this procedure resides on the SESD Local Area Network (LAN). The Document Control Coordinator (DCC) is responsible for ensuring the most recent version of the procedure is placed on the LAN and for maintaining records of review conducted prior to its issuance.

1.4 General Background and Considerations

Characterization of the marine benthic community and associated sediment particle size/chemistry, followed by analysis of community parameters via statistical treatment, may allow for identification and interpretation of changes in the community structure brought about by changes due to ocean dredged material disposal.

Samples are typically collected from either a ship or a boat large enough to go offshore and with enough space and means to deploy sampling equipment and/or divers as well as space to process samples. Marine benthic macroinvertebrate samples may be collected for species identification and associated metrics either by diving or by utilizing a grab sampler such as a Young grab. SESD uses modified VanVeen, (Young) grabs for marine sediment chemistry and macroinvertebrate sampling. These grabs typically have a sampling surface area that ranges from 0.04 - 0.1 m^2 (Figure 1), however any similarly sized sediment grab could be used to collect the sample. A small Young grab has a surface area of approximately 0.04 m^2 and the larger Young grab has a surface area of

approximately 0.1 m^2. The sampling depth is approximately 15 cm. A double grab is preferred, utilizing one side for macroinvertebrates and the other side for sediment chemistry (Figure 2). For most applications, the smaller 0.04 m^2 size is sufficient both in terms of statistical validity and the cost per sample. However, two replicate 0.04 m^2 grabs per station have been shown to greatly increase the species saturation curve and account for some of the spatial variability within a site (Barry Vittor and Assoc., 2015).

When using a grab, the target acceptable grab sample is one that is almost full to the top with a small lens of water over undisturbed sediment. Penetration depth is greatly affected by sediment consistency, with softer sediments allowing deeper penetration of the grab than compacted sand. Therefore, it is very important to monitor the grab penetration depth for every sample and adjust by adding or taking off weight. In very soft sediment, runners made of PVC or wood may be added to the bottom of the grab in order to prevent over penetration.

If samples are collected by diving, the number of cores taken should approximate a similar surface area and volume as samples collected by grabs. One advantage of diver collected cores is that it can account for small scale spatial variability within a site.

Once collected and processed, the benthic analysis macroinvertebrate samples are stored in an alcohol based non-toxic tissue preservative, typically NOTOXhisto®, or other similar preservative. SESD no longer uses formalin as a tissue preservative due to its acute toxicity to humans and listing as a probable carcinogen.

When collecting macroinvertebrates for chemical analysis, a different sampling approach must be utilized due the amount of tissue required for the various analyses. Typically, a minimum of 30 grams of tissue is required for any individual analysis, so for a typical suite of organic and inorganic analyses, 90-120 grams of each type of tissue to be analyzed is required. Collecting this amount of tissue generally requires some type of dredge that is towed behind a vessel for a distance of several hundred yards, typically penetrating into the upper 15-30 cm of substrate. The dredge normally consists of a grated box type frame with a rectangular opening in front and rear (Figure 3). Attached to the rear of the dredge is a retaining net. The dredge normally will have teeth which dig into the sediment, loosening it up and directing it back into the frame or it may have water jets which hydraulically loosen the sediment. The hydraulic dredge will generally provide a more intact sample, but is more difficult to set up and use. Sediment and macroinvertebrates living therein are scooped into the dredge as it is towed along and washed back into the retaining net attached to the dredge.

Macroinvertebrate samples collected for tissue analytical analysis are wrapped in aluminum foil, labeled and frozen for storage and transport to a laboratory facility.

1.5 References

SESD Operating Procedure for Logbooks, SESDPROC-010, Most Recent Version.

SESD Operating Procedure for Field Sampling Quality Control, SESDPROC-011, Most Recent Version.

SESD Operating Procedure for Sediment Sampling, SESDPROC-200, Most Recent Version.

United States Environmental Protection Agency (USEPA). Diving Safety Manual. v 1.3. US Environmental Protection Agency, Washington, DC. Most Recent Version.

USEPA. Safety, Health and Environmental Management Program Procedures and Policy Manual. Science and Ecosystem Support Division, Region 4, Athens, Georgia, Most Recent Version.

Barry A. Vittor and Associates. White Paper, A comparison of Benthic Macroinfauna Sampling Efficiency at Selected Stations in the Fernandina Beach ODMDS, November, 2015. Prepared for Battelle, Inc., Norwell, MA and US-EPA R4, Atlanta, GA.

1.6 General Precautions

1.6.1 Safety

When deploying a grab sampler or dredge from a ship, the vessel's safety protocols for over the side deployment of sampling equipment must be followed. If samples are collected by diving, all diving must be conducted in accordance with EPA's Diving Safety Manual v 1.3 (USEPA). A dive safety plan will be generated by the EPA divemaster in charge of diving on the survey prior to departure. An EPA divemaster must be on site during diving activities. Care must be taken when transferring personnel and equipment from the ship to a small boat or vice versa, especially during heavy seas.

When pouring the NOTOXhisto® or other preservative solution, latex gloves and safety glasses should be worn. Pouring NOTOXhisto® for sample preservation should only be done outside in a well ventilated area or in a vented fume hood. Containers of tissue preservative or preserved samples should be stored within spill containment containers such as large coolers or other containers capable of holding the volume of liquid within should a spill occur.

1.6.2 Procedural Precautions

The following precautions should be considered when collecting samples.

- Documentation of field sampling is done in a bound logbook.
- Chain-of-custody documents shall be filled out and remain with the samples until custody is relinquished.

Figure 1: Young Grab

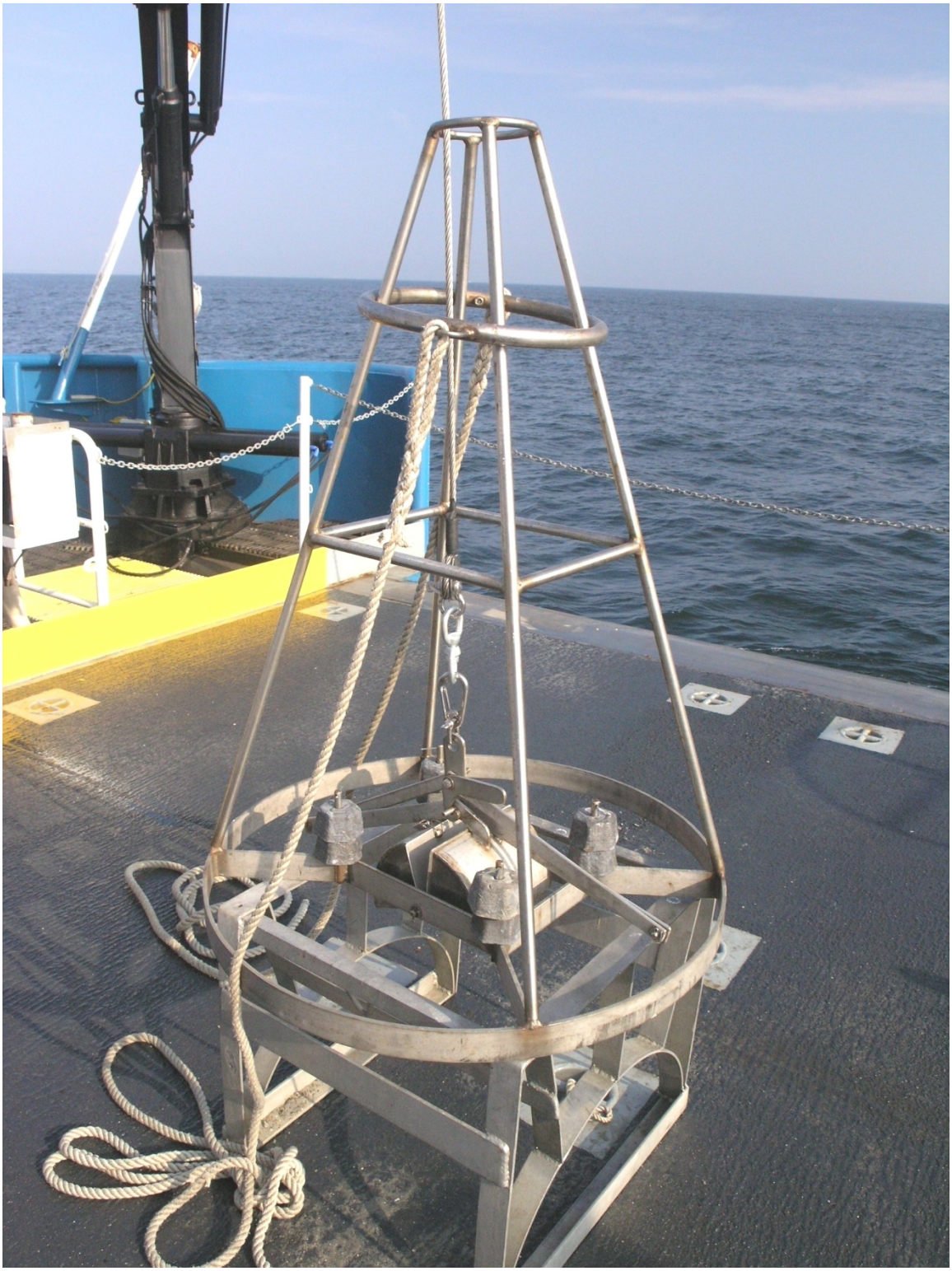

Figure 2: Double Young Grab

Figure 3: Rocking Chair Clam Dredge

2 Marine Benthic Macroinvertebrate Collection

2.1 Macroinvertebrates Collected for Species Identification

Once samples are brought onboard either by diver or by grab, the samples are transferred to a screened (0.5 mm) sieve bucket. A sample may be split into multiple buckets and recombined after sieving in order to expedite the process. The samples are rinsed and washed with ambient sea water either by the raw sea water system aboard the survey vessel or by a 12 volt submersible pump if working from a small boat. All of the sediment smaller than 0.5 mm is washed through the screen leaving the benthic macroinvertebrates and remaining sediment on the screen. The sample retained on the screen after washing is carefully placed in a sample bag, labeled internally and externally, and placed in a 2-5 gallons plastic bucket which has been pre-filled, approximately one-third to one-half full, with NOTOXhisto® or similar non-toxic tissue preservative. The buckets can typically hold several stations worth of samples; therefore, each bucket is labeled with the station names and number of sample reps contained within each bucket. Sample buckets are sealed with a spill proof, rubber gasketed lid and stored in a secondary spill containment container such as a large cooler for transfer to a laboratory facility for taxonomic identification.

2.2 Macroinvertebrates Collected for Tissue Analytical Analyses

Samples being collected by dredge for analytical analyses are brought on board in the collection net attached to the dredge. This is normally very heavy and must be lifted by means of the winch or crane used for deployment. Since samples are being collected for chemical analyses, care must be taken not to contaminate the samples, i.e. only pre-cleaned equipment is used, personnel handling and sorting samples should wear latex gloves and the net containing the samples is laid on several large pieces of aluminum foil in order to avoid contact with the deck. Once on board, the net is detached from the dredge and the sample is divided up into sorting pans in order to pick the appropriate species out of the sample. Bivalves are opened to remove the tissue and polychaetes are removed from their tubes. Once sufficient sample is collected for analysis, it is placed in aluminum foil, labeled and frozen for transport back to a laboratory facility.