

CHAPTER 6

SALTWATER BENTHIC MACROINVERTEBRATES

Disclaimer

Methodologies for assessing ALU have not been developed for Texas saltwater habitats including Gulf waters, bays, estuaries, the Intracoastal Waterway, and tidal streams. Before conducting any biological monitoring activities on a saltwater body, it is imperative to coordinate this work with the TCEQ and the TPWD. As methodologies and metrics are established, this manual will be updated to reflect those changes.

Objective

The objective of this chapter is to describe the methods used by the TCEQ for the collection and assessment of benthic macroinvertebrate samples from saltwater systems. In general, the TCEQ will use benthic macroinvertebrate samples collected according to these methods in conjunction with fish community surveys and physical habitat assessments in an attempt to provide a holistic evaluation of the health of biological assemblages and to develop future indices of aquatic life use for these waters. Sampling saltwater benthic macroinvertebrates also facilitates the collection of data that can be used for assessing water quality trends and comparing water quality differences between sites (USGS 1977).

Years of data collection and a large data set may be needed to develop a reliable estimate of relative abundance for marine and estuarine species. However, if all data from similar saltwater habitats are collected using comparable gears and techniques, the data will be valuable, not only for the given study, but also to the development of assessment methodologies for these salt water bodies.

Any study employing saltwater benthic macroinvertebrate collection must have clearly defined objectives. Careful consideration of the end uses of the data is essential. Methods, gears, and levels of effort must be chosen with the goal of meeting the study objectives. Collections at each site in the study must be comparable. Consequently, collectors must ensure that the sampling procedures, level of effort expended, and types of habitat sampled are similar at each station and in succeeding sample events.

Scientific Collection Permit

Aquatic insects are not protected under state law; however, an SCP is required for the collection of benthic macroinvertebrates. This requirement applies to certain protected native mussels and amphipods, as well as to oysters, shrimp, clams, mussels, and crabs that are subject to license requirements, possession limits, means and methods of take, and size restrictions. If native mussels are included in a benthic macroinvertebrate sample, the collector is encouraged to report this information along with annual fish lists to the TPWD.

Sample Site Selection

Marine macrobenthos are collected from a soft-sediment bottom, rather than from an area with sand, shell litter, oyster reef, or grass flats, unless there is a compelling reason to do otherwise. In choosing a sample location, some trial dredge hauls will help determine an area of suitable silt or mud sediments.

Sampling Procedure

The Ekman dredge is the preferred sampler for collecting benthic macroinvertebrate samples from estuarine habitats. In estuarine areas with large amounts of shell hash or hard sand it may be

necessary to use a heavier dredge, such as a Ponar or Van Veen dredge. Use the same type of dredge at a station to ensure consistency in the data set over time. Before using any of these devices, inspect them carefully to ensure that all parts are in good operational condition. The following collection methods refer to the Ekman dredge but, with only minor exceptions, apply to other dredges as well.

Collect a minimum of four Ekman dredge samples, each placed and preserved in a separate sample container, according to the following procedures:

Before collecting the sample, thoroughly rinse the Ekman dredge in ambient water. Once the Ekman has been cleaned, use the line (or pole in shallower areas) to lower the dredge to the bottom. Avoid lowering the sampler too rapidly as this could cause a pressure wave that can disturb the topmost sediment or give a directional signal to invertebrates capable of retreating from the sample area.

Once the Ekman reaches the bottom, and you have determined that the line is vertical and taut, drop the messenger. After the dredge jaws are triggered, retrieve the closed dredge at a moderate speed (< 1 m/sec). At the water's surface, make sure the jaws are closed and the surface layer of fine silt is intact. Water must cover the sediment sample in the dredge. Do not drain the water off as this may cause the loss of organisms. Bring the dredge on board and empty it into a large container, such as a large plastic tub. Collect the remaining replicates in the same way, placing each into a separate tub.

Sample Washing

To minimize damage to organisms, homogenize the sample by hand. Wash the sediments overboard through a #30 (mesh size ≤ 595 μm) or #35 (mesh size = 500 μm) sieve bucket by dunking the bucket gently, or gently washing with a deck pump. If a deck pump is used, screen the water through a slightly finer mesh than the benthic sieve to inhibit contamination of the sample with plankton.

Narcotizing Sample

Narcotizing the sample 'relaxes' the soft-bodied organisms and may make identification easier. If the sample is to be narcotized, wash the material retained on the bucket screen onto a 0.5 mm sieve, and place the sieve in a suitable bucket with narcotizer to a depth of about 3 cm. The narcotizer must cover the sample without washing the sample out of the sieve. Narcotizer is 7 percent magnesium chloride (MgCl_2) in sea water (75 g of MgCl_2 per liter). After the sample is narcotized for 0.5 to 1.0 hr, remove the sieve from bucket. The narcotizer can then be reused for subsequent samples.

Preserving in the Field

Wash the sample from the sieve or bucket with ambient water into a wide-mouth jar. *It is very important **not** to use freshwater to rinse newly collected benthos.* Preserve in 10 percent formalin (one part full-strength formalin to nine parts seawater). Add several grams of borax to buffer the formalin solution. Do not attempt to use alcohol for a fixative with marine organisms.

Safety Note: Avoid breathing **formalin** fumes! Formalin is corrosive to the eyes, skin, and respiratory tract. Wear safety glasses and latex when working with this suspected carcinogen. Always work in a well ventilated area or under a hood when preparing formalin solutions.

Alcohol is highly flammable. Take care in storage and handling.

Check the alcohol and formalin solution Material Safety Data Sheets for proper handling requirements.

Use adequate preservative to cover the sample. To ensure adequate preservation of benthic macroinvertebrate collections, fill sample containers no more than one-half full with the sample, so the amount of preservative is at least equal to the volume of organic material, including detritus. Avoid placing too much sample in one jar. If there is too much organic matter in the jar, the sample may begin to decompose before processing.

Labeling the Sample Container

Place a label in each sample container that includes, at minimum the following information. Use pencil or waterproof ink on paper with a high rag content for each label.

- Station number and location description
- Date and time of collection
- Collection method (for example: Ekman dredge)
- Preservative used
- Name of collector(s)
- Container replicate number (for example: 1 of 2 or 2 of 2), if needed

Preserving in the Lab

When a preserved sample is brought into the laboratory, it will be assigned a sample tracking number according to the sequence in the benthic macroinvertebrate sample log and logged with pertinent information, including:

- Sample tracking number
- Collection date and time
- Station number and location description
- Name of collector(s)
- Collection method (for example: Ekman dredge)
- Sieve type
- Number of containers in each replicate sample

Safety Note: To reduce your exposure to formalin, rinse the sample with water in a sieve with mesh size $\leq 595 \mu\text{m}$ to remove preservative and fine sediment and transfer to alcohol before sorting. Do this under a ventilation hood or if a hood is not available, in an area with good ventilation.

Within two weeks of sample collection, transfer the samples from the formalin mixture used in the field to a solution of 70 percent ethanol or isopropyl alcohol. To do this, rinse the field-preserved sample with water in a sieve and return it to its original (rinsed) jar, and add 70 percent ethanol or isopropyl alcohol as the new preservative. Rose Bengal vital stain (or another appropriate stain) may be added to the samples to aid in sample sorting. The stain can be added to the formalin-preserved sample in the field or to the alcohol-preserved sample in the lab. Add a small amount (about 0.25 g) of Rose Bengal vital stain and swirl the jar to mix the stain and fixative with the sample.

Sorting in the Lab

The objective of processing an estuarine benthic macroinvertebrate sample in the laboratory is to count and identify every individual benthic macroinvertebrate collected.

Process each of the four replicate samples individually. Place all of the individuals from each replicate grab sample in a separate vial.

Once sorting is complete, there will be four separate vials if four replicate samples were collected, each containing all of the specimens from each individual replicate. Processing each replicate sample separately is important since it will allow the variability between replicates to be evaluated.

Thoroughly rinse the sample using a No. 30 or smaller ($\leq 595 \mu\text{m}$) sieve to remove preservative and fine sediments. Place rinsed sample in a shallow white pan. Put 1 to 2 cm of water in the bottom of the pan to disperse the contents as evenly as possible. Using a lighted magnifying device (2x), pick *all macroinvertebrates* visible from the sample and place in a sample bottle or vial containing 70 percent ethanol or isopropyl alcohol and a label. It is usually necessary to place a small portion of the sample in the pan to ensure that no organisms are missed.

Repeat this process until the entire replicate sample has been inspected under magnification. Most of the organisms will be stained red or pink, but some may be very dark or light red, and some mollusks will not stain well at all.

After thoroughly inspecting the sample and removing all macroinvertebrates, either replace the sample in alcohol for later checking or have another investigator check to ensure no organisms were missed. Check at least 10 percent of samples for missed organisms. Record the date and identity of the sorter(s) in the sample tracking logbook.

Labeling a sample. Label the sample bottle or vial containing the sorted and counted benthic macroinvertebrates with the following information. Use pencil or waterproof ink on paper with a high rag content for each label.

- Station number and location description
- Sample tracking number
- Date and time of collection
- Collection method (for example: Ekman dredge)
- Container replicate number (for example: 1 of 3, 2 of 3, 3 of 3)
- Preservative used
- Name of collector(s)
- Name of person conducting sorting procedure, if different from collector

Repeat this labeling process for each of the four replicate samples.

Additionally, affix a label with the sample tracking number and container replicate number to the outside of the container—but *never on the lid*. Make sure the container is dry, and wrap with clear tape to ensure the label will not come off.

Laboratory Procedures for Identification of Specimens

Identification and enumeration of estuarine benthic macroinvertebrates must be conducted by individuals with appropriate expertise, training, and knowledge of the literature. Using appropriate reference(s), a stereo dissecting microscope, and compound microscope, identify the organisms to the lowest practical taxonomic level—*species* in most cases. Chapter 11 provides a complete listing of required and recommended saltwater benthic macroinvertebrate identification references. Record the species names and counts for each replicate on a laboratory bench sheet that contains the sample information recorded on the label. *Maintain a separate count of individuals and list of taxa for each replicate grab sample to allow an evaluation of variability between replicates.*

Records

In addition to sample labeling requirements as specified in this chapter, maintain the following records.

Field logbook. For each sample event, record all relevant information in the field logbook, including the date and time of sample collection, location of the sample site (Station ID), name(s) of collector(s), method of collection, number and type of samples collected, number of sample containers, and preservative used.

Sample tracking logbook. Maintain a sample tracking logbook that contains the information described in the QA chapter of this manual. This logbook documents when samples arrive at the

laboratory or headquarters facility, when each sample enters each sample processing step, and who has custody or responsibility for the sample.

Laboratory bench sheets. Maintain laboratory bench sheets at the location where specimen identification and enumeration occurs. These bench sheets document the raw counts of individuals for each taxon and notes relevant to the identification and enumeration process.

Voucher Specimens

Retain the benthic macroinvertebrates collected as voucher specimens for a minimum of five years or until the conclusion of applicable regulatory decision (whichever is longer) to allow identification verification if necessary. Voucher specimens serve as long-term physical representations that substantiate the names applied to organisms collected as part of the TCEQ SWQM program. Voucher specimens ensure the credibility of TCEQ bioassessment data by documenting the identity of the organisms and making them available for review by the general scientific community.

Take the following into consideration when storing voucher specimens:

- Long-term maintenance of wet (alcohol-preserved) and mounted specimens
- Adequate quantity and quality of space to store specimens
- An effective mechanism for locating and retrieving specimens upon request
- Personnel experience in invertebrate taxonomy

The organization maintaining voucher specimens must have a history that indicates it will be able to preserve the specimens into the future (USGS 2000). This could include in-house provisions for maintenance of samples or archiving at a natural history collection at a university.

Tidal Streams

In tidal streams or estuaries with sandy bottom sediments, a Van Veen or Ponar dredge might be necessary to collect benthic macroinvertebrates. A suction-coring device is another alternative for collecting a good sample from some locations.

