

## CHAPTER 6

# SALTWATER BENTHIC MACROINVERTEBRATES

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## Disclaimer

Methodologies for assessing ALU and other regulatory bioassessments have not been developed for Texas saltwater habitats, including Gulf waters, bays, estuaries, the Intracoastal Waterway, and tidal streams. Before any biological monitoring of 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

This chapter describes the methods used by the TCEQ for the collection and assessment of benthic macroinvertebrate samples from saltwater systems. In general, the TCEQ will use samples collected according to these methods in conjunction with fish-community surveys and physical habitat assessments to holistically evaluate the health of biological assemblages and to develop future indices of aquatic-life use for these waters. Sampling saltwater benthic macroinvertebrates derives 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 kinds of gear and techniques, the data will be valuable, not only for the given study, but also to the development of assessment methodologies for saltwater bodies.

Any study employing the collection of saltwater benthic macroinvertebrates must have clearly defined objectives. Careful consideration of the data uses is essential. Choose methods, kinds of gear, and levels of effort with the goal of meeting study objectives. Sample collections at each site in a study must be comparable. Consequently, collectors must ensure that the sampling procedures, effort, and types of habitat sampled are similar at each station and in succeeding sampling events.

## Scientific Collection Permit

An SCP is required for the collection of marine benthic macroinvertebrates. This requirement applies to oysters, shrimp, clams, mussels, and crabs that are subject to license requirements, possession limits, means and methods of take, and size restrictions.

## Equipment

Field equipment and materials necessary to conduct freshwater benthic macroinvertebrate sampling appear in Appendix A.

## Selecting the Sampling Site

Marine benthic macroinvertebrates 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 sample those habitats. In choosing a sample location, some trial dredge hauls will help determine if an area has 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, a heavier dredge may be necessary, 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 it 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 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 that 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 No. 30 (mesh size  $\leq 595 \mu\text{m}$ ) or No. 35 (mesh size =  $500 \mu\text{m}$ ) sieve bucket by dunking the bucket gently, or gently washing it with a deck pump. If using a deck pump, 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 narcotizing solution must cover the sample without washing the sample out of the sieve. Narcotizing solution is 7 percent magnesium chloride in seawater—75 g of  $\text{MgCl}_2$  per liter.

After 0.5 to 1.0 hour the sample is narcotized and the sieve can be removed from the 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 it 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 use alcohol as a fixative with marine organisms.

Use enough preservative to cover the sample. To ensure adequate preservation of benthic macroinvertebrate collections, fill sample containers no more than 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 and limit the amount of water when transferring sample material to the jar. Too much organic matter in the jar or not enough preservative can cause samples to start decomposing before they can be sorted and identified.

## **Safety**

Avoid breathing formalin fumes! Formalin is corrosive to the eyes, skin, and respiratory tract. Wear safety glasses and latex gloves 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 material-safety data sheets for alcohol and formalin for proper handling requirements.

## ***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 each collector
- container replicate number if needed (for example, *1 of 2* or *2 of 2*)

## **Sample Processing**

### ***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 each collector
- collection method (for example, Ekman dredge)
- sieve type
- number of containers in each replicate sample

Within two weeks of their 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. You may add Rose Bengal vital stain (or another appropriate stain) to the samples to aid in their 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 preservative with the sample.

## **Safety**

To reduce formalin exposure, 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. Follow your organization's hazardous waste disposal plan for formalin waste.

## ***Sorting in the Lab***

The objective of processing an estuarine benthic macroinvertebrate sample in the laboratory is to count and identify every individual 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 allows 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 the 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 (2 $\times$ ), pick **all visible macroinvertebrates** and place them in a sample bottle or vial containing 70 percent ethanol or isopropyl alcohol and a label. It is usually necessary to place small portions 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 that no

organisms were missed. Check at least 10 percent of samples for missed organisms. Record the date and identity of each sorter in the sample-tracking logbook.

## ***Labeling a Lab 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 each collector
- 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. Make sure the container is dry, and wrap it with clear tape to ensure the label will not come off. Do not put the label on the container lid.

## **Identifying Specimens in the Laboratory**

Identification and enumeration of estuarine benthic macroinvertebrates must be conducted by persons with appropriate expertise, training, and knowledge of the literature. Using appropriate references, a stereo dissecting microscope, and a compound microscope, identify the organisms to the lowest practical taxonomic level, **species** in most cases. Chapter 11 gives a complete list of required and recommended references for identifying saltwater benthic macroinvertebrates. 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***

Besides the labeling of samples specified in this chapter, maintain the following records.

### **Field Logbook**

For each benthic macroinvertebrate sample event, record the following in a field logbook.

- date and time of sample collection
- the location of the sample site (station ID)
- name of each collector

- method of collection
- number and type of samples collected
- number of sample containers
- preservative used

## **Sample-Tracking Logbook**

Maintain a logbook that documents when samples arrive at the laboratory or headquarters, the steps in processing samples, and who has custody or responsibility for each sample.

Upon returning to the laboratory, assign a unique sample tracking number to each jar containing the fish specimens according to the sequence in the fish sample-tracking logbook. For example, an instance of numbering may look like *BM 040 14*, where *BM* refers to ‘benthic macroinvertebrate,’ *040* refers to sample number 40, and *14* refers to the year 2014.

Record the tracking number and related information on the sample in the logbook. This information includes:

- sample tracking number
- date and time of collection
- station number and location description
- name of each collector
- collection method (for example, kicknet or snag)
- preservative used
- number of containers in sample

## **Laboratory Bench Sheets**

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

## ***Voucher Specimens***

Retain the benthic macroinvertebrates collected as voucher specimens for at least five years or until the conclusion of any applicable regulatory decision (whichever is longer) to allow verification of identification if necessary. Voucher specimens serve as long-term physical proof that confirm the names applied to organisms stored in SWQMIS. Voucher specimens ensure the credibility of TCEQ biological 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 sample maintenance of samples or archiving in a university or museum natural-history collection.

### ***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.

