Benthic Invertebrate Standard Operating Procedures

USGS Western Ecological Research Station SFBE

Sieving methods were modified from: US Geological Survey. 2010. Invertebrate lab manual. Unpublished benthic invertebrate sieving and sorting protocols. USGS, Western Ecological Research Center, San Francisco Bay Estuary Field Station, Vallejo, CA.

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Purpose/Objective:
Invertebrates convert decomposing marsh plants and detritus into biomass available to fish and avian communities at higher trophic levels. As such, invertebrates are useful early monitoring indicators of the success of the estuary restoration project.

These protocols provide information on sample collection, sieving and preservation. After preservation, samples will be sent to a qualified invertebrate laboratory for sorting, identification, enumeration and weighing for dry biomass. If you decide to sort and identify in-house, refer to the *Invertebrate Lab Manual* (USGS 2010) for details on sorting and identification.

Equipment:

Collection

- Sediment corer (a.k.a. Clam gun)
- Ziploc bags (1gallon) bring extras
- GPS
- Aerial Photo with core locations
- Waders
- Backpacks
- Flexible spatula
- 5 gallon buckets (3-4)
- Water Quality Spot Meter (e.g. YSI)
- Sample Data Tracking Sheet
- Mud shoes (if needed)
- Cooler (to keeps cores cold)

Sieving

- Sample Data Tracking Sheet
- Number 35 (0.5 mm) sieve
- Pencils, black thick sharpie
- Labels
- 16 oz. Nalgene squirt bottles
- 4 or 8 oz polystyrene jars, lids
- Dissecting forceps, spatula
- Bottomless bucket (same diameter as sieve)
- Cooler (to keeps cores cold)
- 70% Ethyl alcohol with Rose Bengal dye (Be careful-read MSDS)

Methods:

Collection

1. Inverts are collected at low tide when the channel bottom is exposed; the tide level at which this occurs will vary from site to site.
    a. Sediment cores will be taken with a 10 cm diameter corer to a depth of 10 cm.
    b. After inserting corer 10 cm, place a finger on the hole just underneath the handle to create suction.

c. Lift core and dump into **labeled** plastic bag (label with site, date, core#, on the outside and with a Rite in the Rain label on the inside). *Note: Due to muddy conditions, it is better to label bags before going out into the field.*
d. Let air out of bag and seal securely.
e. Place in bucket or backpack. (Try to limit inverts from direct sunlight and heat for long durations. If needed, take to truck and place in a cooler in the shade.)
f. Store samples in the refrigerator (NOT FREEZER) prior to sieving. Samples must be sieved and stored in 70% ethanol-rose Bengal solution **within one week of collection**.

2. The number of replicates will be determined by study design. An additional core will be collected and sent to a lab to analyze for sediment characteristics (pore-water soil salinity, particle size, organic content, nutrients, etc.).

3. Use handheld multi-probe to measure water quality (temperature, salinity, dissolved oxygen, and conductivity) readings at each sampling station.

SievingSamples must be sieved and stored in 70% ethanol-rose Bengal solution **within one week of collection**. Samples with large amounts of clay and silt may need to be re-rinsed and re-sieved prior to sorting, in order to help break up large lumps of clay and ensure adequate preservation of specimens.

1. If the sample contains a lot of silt or clay, add a small amount (approximately 0.1 g) of sodium hexametaphosphate ((NaPO3)6) to samples, gently shake and allow to sit for at least 12 hours.

2. Gather a 0.5 mm sieve, the data sheet, and take your samples to a hose or utility sink. If sieving many samples, keep core samples in a cooler or fridge until sieving.

3. Pick a sample to process and examine the contents. Make sure any samples needed for sediment analysis are removed before sieving. Start a new datasheet for each sample location and record the following:

   At top of data sheet:
   Project name, location within project, date of collection, number of cores, and names of collectors

Project Name:				Location:			Type: Benthic
Number of Cores:				Collected by:			Collection date:
Sample		Sieving		Sorting			
Core	Rep	Sieved on	By	Sorted on	By	# of Vials	Notes

   For each sample:
   Sample ID, initials of siever, and date of sieving

Project Name
Sample ID
Collection Date
Siever initials & Date

4. Place the samples in a bucket of clean water. The largest, heaviest sediments will settle to the bottom. Pour the water with suspended invertebrates gently over the sieve.

5. Scan the sieve and pick any invertebrates from the sieve and place into a labeled 40 mL vial containing 70% ethanol for preservation.

6. Repeat this process until the core is completely broken up and rinsed into the sieve. Place the remaining sample matrix into labeled jars, making sure jar is labeled on both the side and lid, and a rite-in-the-rain label is placed inside the jar. Add solution of ethanol and rose Bengal dye solution.
   * Note: Samples with a large amount of organic matter require a higher concentration of ethanol (95%) for adequate preservation.

How to Make Rose Bengal 70% Ethanol Solution

$C_i * V_i = C_f * V_f$

$0.95 * V_i = 0.70 * 4{,}000$ mL

V_i = 2947 mL of 95% EtOH

4000 mL (V_f) – 2947 mL (95% EtOH) = 1,053 mL of DI H_2O

So, add:

2,947 mL of 95% Ethanol

1,053 mL of distilled water

Small spatula of Rose Bengal

Sorting and Identification

After preservation, samples will be sent to a qualified invertebrate laboratory for sorting, identification, enumeration and weighing for dry biomass. Abundances will be standardized to area and reported as average density of invertebrates per m^2. If you decide to sort and identify in-house, refer to the *Invertebrate Lab Manual* (USGS 2010) for details on sorting and identification.

When monitoring is focused on invertebrates as prey resources, invertebrate identification to the lowest taxonomic level, although informative, may not be cost effective to answer questions based on prey resources. Rather, the taxonomic categories of interest should consider the known diet of predators of interest and their foraging modes or behavior.

Data Entry and Analysis:
Benthic invertebrate data can be used in multiple analyses. Examples include:

1. Analyzing change in invertebrate composition over time in regards to restoration actions (Figure 1).

2. Comparison of restoration invertebrate composition to reference sites (Figure 2).

3. Correlation analysis between invertebrate composition and environmental variables (e.g. water quality, elevation, soil characteristics).

4. Calculation of available prey resources to fish and avian communities.

5. Use in fish diet analyses when fish diet data has been collected (e.g. percent similarity indices between stomach contents and available prey resources).

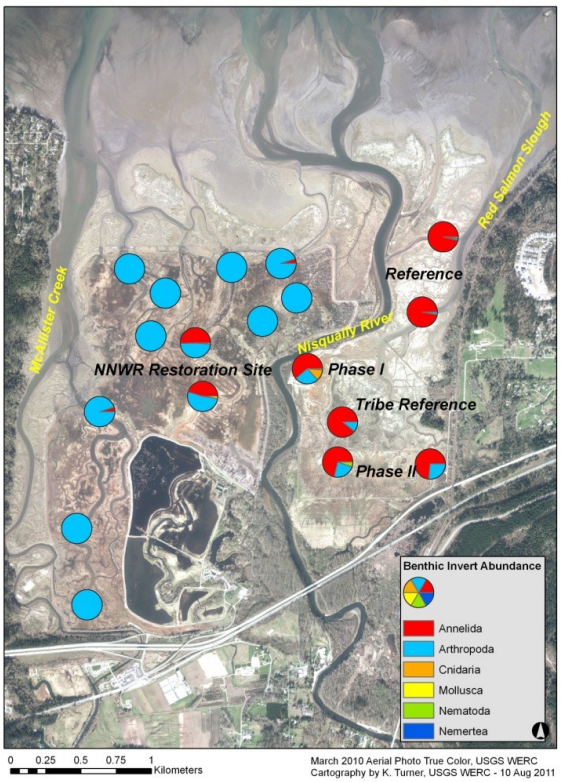

Figure 1. Proportional abundance of major benthic invertebrate taxa, pre-dike removal, 2009, Nisqually estuary. These data can be compared to post-dike removal surveys.

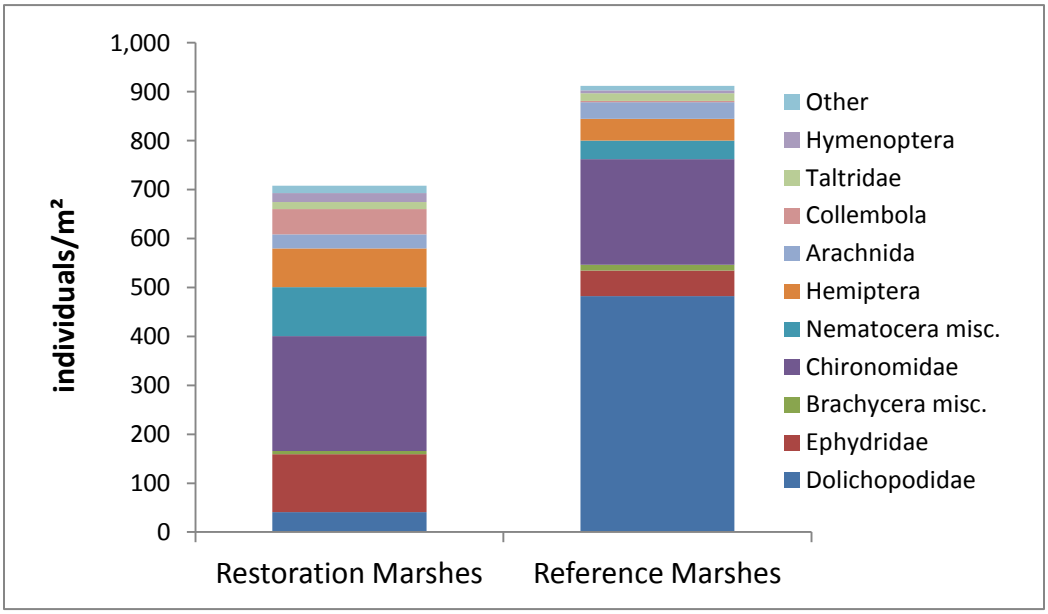

Figure 2. Average density by taxa at restoration and reference marshes in 2010, Nisqually estuary.