Washington State Department of Ecology

Environmental Assessment Program

Standard Operating Procedures for Benthic Macrofaunal Size Classification and Biomass

Version 1.1

EAP126

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Any reference to specific equipment, manufacturer, or supplies is for descriptive purposes only and does not constitute an endorsement of a particular product or service by the author or by the Department of Ecology.

Although Ecology follows the SOP in most instances, there may be instances in which the Ecology uses an alternative methodology, procedure, or process.

SOP Revision History

Revision Date	Rev number	Summary of changes	Sections	Reviser(s)
3/30/2017	1.0	Review and initial certification	all	Bill Kammin
4/3/2017	1.0	Cover page, footer	all	Bill Kammin
10/13/2017	1.1	Changes to size class definitions	3.5-3.7, 6.6.1	Dany Burgess
10/13/2017	1.1	Changes to subsampling procedure	6.6.8	Dany Burgess
10/13/2017	1.1	Updates to procedure for dealing with fragments of organisms	6.6.9	Dany Burgess
10/13/2017	1.1	Changes to size class determination	6.6.10	Dany Burgess

1.0 Purpose and Scope

1.1 This document is the Environmental Assessment Program (EAP) Standard Operating Procedure (SOP) for the determination of size classification and biomass of benthic macrofaunal invertebrates, collected in accordance with SOP EAP039. This SOP covers field and laboratory methods for obtaining size measurements and wet weights of macrofaunal organisms, establishment of a size class reference collection, new procedures for taxonomic identifications, and data recording. These methods follow those recommended by the Vancouver Aquarium's Coastal Ocean Research Institute (CORI) as part of the Salish Sea Ambient Monitoring Exchange (SSAMEx) project.

1.2 Personnel from Ecology's Marine Monitoring Unit will lead all sample collection and processing.

2.0 Applicability

2.1 This SOP should be followed for all Puget Sound Ecosystem Monitoring Program (PSEMP) Marine Sediment Component macrobenthic sample analysis performed by Ecology's Marine Monitoring Unit or selected vendors.

3.0 Definitions

3.1 Benthic macroinvertebrate sample – The entire contents of one side of the grab sampler that is collected for identification and enumeration of macro invertebrates residing in the sediment.

3.2 Biomass - The amount of living matter present at any given time, expressed as mass per unit area or volume of habitat.

3.3 Wet weight – Refers to the weight of animal tissue including its contained water.

3.4 Size class – arbitrary grouping of individuals of a species which fall into a pre-determined range of measurements and developmental characteristics.

3.5 Small (Size Class) – Benthic macroinvertebrates that are generally juveniles, immature specimens in early stages of development, or the smallest intermediates/subadults.

3.6 Medium (Size Class) – Benthic macroinvertebrates that may be small adults; have adult features and are still identifiable to the same level but have not attained the typical adult body size.

3.7 Large (Size Class) – Benthic macroinvertebrates with fully-developed adult features, typically the largest specimens encountered (with the exception of megafauna); weigh ≤ 2 g.

3.8 Megafauna (Size Class) – Benthic macroinvertebrate weighing more than 2 g.

3.9 MSDS – Material Safety Data Sheets provide both workers and emergency personnel with the proper procedures for handling or working with a particular substance. An MSDS includes information such as physical data (melting point, boiling point, flash point, etc.), toxicity, health effects, first aid, reactivity, storage, disposal, protective equipment and spill/leak procedures.

4.0 Personnel Qualifications/Responsibilities

4.1 All staff must comply with the requirements of the EA Safety Manual (EA Program2006). A full working knowledge of the procedures in Chapter 2 section 2-7 "Handling Formaldehyde" and section 2-31 "Rescreening Marine Benthic Samples Preserved with Formaldehyde" is expected.

4.2 All staff must be familiar and comply with the requirements of Ecology's Chemical Hygiene Plan and Hazardous Materials Management Plan.

4.3 Rescreening, sorting, and taxonomic identification of benthic invertebrates requires the use of 37% formaldehyde (formalin), 70% ethanol, glycerol, and various vital stains, each of which can harm workers if used without proper precautions and adequate controls. Before handling, storing, or using these chemicals, each employee must:

4.3.1 Read this standard operating procedure and discuss any questions with his/her supervisor or task team leader.

4.3.2 Read the Material Safety Data Sheets (MSDS) for formalin, ethanol, glycerol, rose bengal, methylene blue, methyl green, and crystal violet, before beginning the sorting/taxonomic procedures. These MSDS are available in the Ecology Headquarters benthic laboratory.

4.3.3 Immediately report to supervisor any symptoms or reactions that might be related to exposure.

4.3.4 Use proper protective clothing and equipment.

5.0 Equipment, Reagents, and Supplies

5.1 Field Equipment and Supplies

5.1.1 Spring scale
5.1.2 Weigh basket
5.1.3 Flexible Measuring Tape
5.1.4 Paper towels
5.1.5 Field Biomass Data sheet
5.1.6 Pencil

5.2 Laboratory Equipment, Reagents, and Supplies

5.2.1 Glass petri dish
5.2.2 Clear plastic ruler
5.2.3 Clear tape
5.2.4 Flexible Measuring Tape
5.2.5 Forceps
5.2.6 Laboratory scale
5.2.7 Stereo dissection and compound microscopes (magnification up to 1,000x)
5.2.8 Microscope light source
5.2.9 Cover slips
5.2.10 Labels (Rite-in-the-Rain® paper)
5.2.11 Vials and Polyseal® caps
5.2.12 Small scalpels
5.2.13 Scissors
5.2.14 Cotton
5.2.15 Petri dishes
5.2.16 Glycerol
5.2.17 Methylene blue
5.2.18 Methyl green
5.2.19 Crystal violet
5.2.20 Latex or nitrile gloves
5.2.21 Ethanol (70% , aqueous), in squirt bottles:
http://www.sciencelab.com/msds.php?msdsId=9923955

6.0 Summary of Procedure

6.1 Field Procedures

6.2 Follow established EAP procedures for obtaining marine sediment samples (SOP EAP039), through section 6.7.5.

6.3 These next steps will serve as an addendum to/replacement for SOP EAP039 section 6.7.6, regarding the field identification and release of megafaunal organisms. Some megafaunal organisms are easily identified in the field and may therefore be released after taxonomic identification, wet weight, and size measurements are collected. Workup and release of megafauna should take place while the sampling vessel is still on station to minimize holding time and avoid transporting animals to other areas of Puget Sound (Parsons et al., 2016).

6.3.1 Identify megafaunal organism and record species name on the data sheet. If there is more than one organism of a single species, record each individual on a separate line. Large polychaetes, some bivalves, and other megafaunal organisms with questionable identifications should be excluded from field ID/measurement and preserved with the rest of the sample to avoid misidentification.

6.3.2 Obtain taxon-specific measurements as specified below and record on the data sheet. Using the measuring tape or ruler, measure length AND width/circumference for all specimens (to the nearest mm).

Stylatula elongata: 1. Length; 2. Width at mid-point of "base"
Molpadia intermedia: 1. Length; 2. Circumference at mid-point
Crabs – 1. Length (anterior margin to abdomen); 2. Carapace width at widest point
Bivalves – 1. Length (longest distance between anterior and posterior shell margins); 2. Width at widest point

6.3.3 Obtain live wet weight of the specimen. Blot specimen for 30 seconds with a paper towel, place into weigh basket, and then air-dry for 1.5 minutes[1].

6.3.4 Attach weigh basket to spring scale. Record wet weight on data sheet to as much resolution as the scale will provide. Weights are in grams on the data sheet; measurements will later be converted to mg when data are entered into the database.

6.3.5 Gently release megafaunal organism(s) in a location as close to the original collection site as possible.

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[1] From Vancouver Aquarium's Coastal Ocean Research Institute's (CORI) SSAMEx proposal: The 1.5-minute waiting period was selected in 2001 after comparing the results of repeatedly weighing organisms using various methods. It was determined that a 1.5-minute waiting period before weighing was unnecessary for smaller organisms.

6.4 Laboratory Procedures

6.5 Follow the Benthic Macroinvertebrate Sample Analysis procedures detailed in SOP EAP043 for Rescreening Infaunal Samples Preserved with Formalin (section 9.1), Sample Tracking (section 9.2), Sample Sorting (9.3), and Sample Tracking and Labeling of Sorted Samples (section 9.4).

6.6 The following sections will serve as a replacement for/addendum to SOP EAP043 section 9.5, Taxonomic Identification of Organisms. As part of the identification process, each organism will be assigned to one of four size categories and will receive a corresponding biomass estimate. These techniques were adapted from methods described in the SSAMEx proposal (CORI, 2015), and are used in the following publications: Burd et al., 2012a, b, 2013, 2014, and Macdonald et al., 2012a, b. The methods described in these studies include 3 size class designations, but methods are currently being revised to include a 4th category for megafauna (Tara Macdonald, personal communication). The methods outlined below reflect this change.

6.6.1 **Establishment of a Puget Sound Benthos Size Class Reference Collection (referred to hereafter as "Reference Collection")**. This will be done in full (for all taxa and size groups) the first time a survey is done in a region, if there are no previous size class reference collections available. For the Marine Sediment Monitoring program, the initial reference collection will be established using the 2016 PSEMP Long-Term benthos samples (22 stations with 3 replicates each; total n = 66) and will apply to all other subsequent sediment monitoring projects in the Puget Sound region. The Reference Collection will include not only verified specimens identified to the lowest possible taxonomic level, but four size classes (small, medium, large, and megafauna; see details below) for each taxon, assigned according to their size and stage of development based on best professional judgement. These are not necessarily developmental categories, but instead refer to the size of the organism:

Small (S): May include juveniles, immature specimens in early stages of development, and the smallest intermediates/subadults. Generally 1/3 the size of those classified as large (see below).

Medium (M): May be small adults or 'subadults'; have adult features and are still identifiable to the same level but have not attained the typical adult body size. Generally about 2/3 the size of those classified as large (see below).

Large (L): The largest specimens of each taxon (with the exception of megafauna); weigh ≤ 2 g.

Megafauna (M): All specimens weighing >2 g.

6.6.2 Sort everything in the sample by taxon. Identifications will be done by in-house taxonomists, using minimum 10x magnification dissecting light microscopes and

compound light microscopes equipped with 4x, 10x, 20x and 40x and 63x magnification objective lenses.

Identification and enumeration of sorted organisms will be performed to the lowest taxonomic level possible, usually to species. If an organism cannot be identified to species level, the taxonomist must decide whether or not to exclude it from data analysis in order to prevent taxonomic redundancy. Exclusions (and corresponding explanatory comments) will be recorded in the database. This decision will be based on rules established by SCCWRP (2013):

Exclude a taxon if ALL of the following are TRUE:

1.) The ID is not at species level (e.g. family or genus).
2.) The taxon is represented in the sample by other members of the same taxon which have been identified at lower levels (e.g., both *Dipolydora socialis* and *Dipolydora* sp. are present in the same sample).
3.) The taxonomist cannot determine if the specimen is distinct from the other members of its taxon in the sample. (In the example above, it is possible that the *Dipolydora* sp. specimen may actually be *D. socialis*.)

A taxon with a higher level of identification **SHOULD NOT be excluded** if there are no other members of that taxon identified at lower levels in the same sample, or if the taxonomist is confident that it is not the same species as those identified at lower levels.

Taxa known to be distinct in the sample but left at higher levels of identification because they are awaiting consultation with a regional expert for ID assistance **SHOULD NOT be excluded**; these IDs will generally be changed to lower levels of identification in the database once the specimens are returned from QA/QC.

6.6.3 Each taxon will go into a separate cryoseal vial with an internal label with the survey name, station ID, collection date, field screen size, taxonomic identification, taxonomist name or initials, and identification date. The label must be placed into the vial before any animals are added, to prevent damage to the animals. The labels will be written on 100% waterproof rag paper (e.g., Rite-in-the-Rain™), using an HB graphite pencil.

6.6.4 Measure the total length (mm) and width at widest point (mm) of each individual animal in the sample, according to the method best suited for that taxonomic group. For most organisms, a glass petri dish with a ruler taped to the bottom can be used to obtain length and width measurements to the nearest mm. For large cylindrical animals like holothuroideans and anthozoans, the length and circumference at the widest point can be taken using a measuring tape. Record species name and measurements into the Taxon Size Classes table in the database.

6.6.5 Obtain a corresponding wet weight for each individual animal that was measured in section 6.6.4. Wet weights are performed with a balance that is accurate to 0.1 mg. If taxa are beyond detection limits, these are reported as 0.00005 mg. Prior to weighing, organisms are removed from ethanol, blotted on a Kimwipe or filter paper, and the first

weight upon placement in the balance recorded. One cannot wait for stabilization of the balance reading as ethanol evaporates quickly, and small organisms will dry out. Record wet weight in the Taxon Size Class table, next to the corresponding measurements.

6.6.6 Polychaetes are typically weighed without tubes. However, some taxa are particularly difficult to remove from tubes without damaging the specimen(s). The same applies for other organisms that inhabit tubes or empty shells (e.g., phoronids, hermit crabs, etc.). In these cases, wet weight for a given taxon should specify whether there are tubes included or not, and be taken consistently using this method for every specimen of that taxon. This information should be compiled in a spreadsheet for future reference.

6.6.7 Megafaunal organisms should be blotted for 30 seconds, placed on the balance pan, and then air-dried for 1.5 minutes (to allow some of the alcohol to evaporate) before the weight is recorded.

6.6.8 **Subsampling**: For species with a large number of individuals in a single sample (>10), obtain weights for a subsample consisting of 10 individuals (this subsample should capture a range of sizes if possible). If there are more than 10 individuals of a species for which existing data are scarce, all individuals in the sample may be weighed and measured.

Once the number of measurements for each size class of a given taxon has reached 150, weighing and measuring of specimens from that taxon may be performed on a randomly selected single replicate from each station, rather than on all three replicates. However, care should be taken to ensure that each size class is well-represented in the data. Under-represented size classes may be targeted in the 1 replicate that is processed, but measured individuals should still capture a range of sizes (i.e., all size classes in the sample should be represented by at least one measured individual).

6.6.9 **The following types of individuals should NOT be weighed or measured and should NOT be included in the Reference Collection:**

1) **Fragments of solitary organisms** (for example, anterior fragments of polychaetes). However, fragments should be used to estimate total body size of the organism, and this estimate used to place the individual into a size class. Wet weights for fragments will be determined by using the average wet weight for the corresponding size class from the reference collection. Exceptions may be made if specimens are nearly complete (e.g. a polychaete missing only one or 2 posterior segments) and there is little or no data available for that taxon; these decisions should be made based on best professional judgement. If a fragment is measured and weighed for biomass, its complete length and weight may be estimated from the length/weight/width of the fragment. Any estimated data associated with incomplete specimens should be noted in the database, and deleted once more complete specimens are found.

2) **Colonial or epifaunal organisms** such as: colonial hydroids, bryozoans, sponges, colonial tunicates, etc., due to likelihood that the organism is only a fraction of a whole colony of indeterminate size. These types of organisms will not be assigned a size class and will not receive a wet weight estimate. Solitary members of these groups, including solitary tunicates and anemones, may be measured, weighed, and assigned a size class.

6.6.10 Once measuring and weighing is completed for the 2016 PSEMP Long-Term samples, determine maximum lengths, or cutpoints, for each size class, for each taxon. Length cutpoints for size classes will be determined using a "rule of thirds", in which the maximum length attained by that taxon is divided into 3 equal parts to obtain the ranges for small, medium, and large classes (the megafauna class has no maximum size and therefore does not need a cutpoint). Maximum length for each taxon will be estimated using a combination of the size range in the literature, the observed size of measured specimens in the Reference Collection, and best professional judgement. Occasionally, taxa do not adhere well to length cutpoints and the widths and weights may be taken into account when choosing a size class.

Using the size class cutpoints and the lengths recorded in section 6.6.4, assign each organism entered in the Taxon Size Classes table to one of the four size classes listed in section 6.6.1, and record the size class abbreviation. Organisms identified to higher taxonomic levels such as genus or family will also be assigned to a size class based on these categories.

6.6.11 Calculate the mean (arithmetic average) wet weight for each of the four size classes within a taxon using the following equation: Sum of wet weights/# individuals weighed. Record the mean wet weight for each size class/taxon. These averages will be used to calculate a total estimated wet weight biomass for each size class/taxon.

6.6.12 For subsequent sampling events, organisms will be assigned to a size class based on the size ranges previously established by the reference collection. It may be necessary to take actual measurements of individual organisms if the size class cannot be determined by sight. Mean wet weights will be used in place of actual wet weights during subsequent sampling events (individuals organisms will not need to be weighed). These rules apply only to the small, medium, and large size classes - megafaunal organisms should always be weighed and measured due to the wide variability in the size of these organisms.

6.6.13 The reference collection should be updated/added to following each subsequent sampling event. Any new taxa encountered should be weighed and measured as described above. Reference specimens for existing taxa should also be added if size classes change from one year to the next based on taxonomist observations.

7.0 Records Management

7.1 A new field data sheet for recording wet weight biomass will be started at the beginning of each sampling season. Record project name/year at the top of the sheet: Y:\SHARED Files\MSMP\Biomass\Field Data Sheet_Megafaunal Biomass.xlsx.

7.2 Laboratory measurements, weights, size class, and comments will be entered directly into a database: currently Y:\SHARED Files\MSMP\Infaunal sample tracking\PSEMP Taxonomy data entry.accdb.

8.0 Quality Control and Quality Assurance Section

8.1 Refer to SOP EAP043, section 10.2.1 for taxonomic quality control measures. This SOP requires that 5% of identified samples be sent out to regional taxonomic experts for re-identification as a QA/QC measure. The separation of taxa into labeled vials by primary taxonomists for the Puget Sound Benthos Size Class Reference Collection (see section 6.6 of the current SOP) will necessitate the masking of species identities for those samples selected to be sent off for QA/QC. Species may be kept in separate vials but the identifications on the internal labels should be replaced with a number or taxon code, such that the species name is known to the primary taxonomist but not the secondary (QA/QC) taxonomist.

8.2 The Puget Sound Benthos Size Class Reference Collection (see section 6.6.1) compiled during the first round of macrofaunal sample processing (2016 PSEMP Long-Term samples) will serve as quality control for biomass estimates, and will be updated as necessary to reflect any changes in the observed size ranges of benthic organisms.

9.0 Safety

9.1 Ethanol (100% and 70% aqueous solution)

9.1.1 Ethanol is a highly flammable, clear liquid with a pleasant odor, and is used for longterm preservation of benthic invertebrates. Ethanol may cause liver damage, may affect the central nervous system, and can cause respiratory tract, skin and eye irritation. User should avoid contact with eyes, skin, and clothing, and wash hands thoroughly after handling. The MSDS for ethanol may be found at http://www.sciencelab.com/msds.php?msdsId=9923955.

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