



DEPARTMENT OF
ECOLOGY
State of Washington

Standard Operating Procedure EAP043, Version 1.4

Marine Macrobenthic Sample Analysis

Jan 2023
Publication 23-03-232
[Recertified 2021]

Purpose of this document

The Washington State Department of Ecology develops Standard Operating Procedures (SOPs) to document agency practices related to sampling, field and laboratory analysis, and other aspects of the agency's technical operations.

Publication Information

This SOP is available on the Department of Ecology's website at <https://apps.ecology.wa.gov/publications/SummaryPages/2303232.html>.

Ecology's Activity Tracker Code for this SOP is 12-075.

Recommended citation:

Burgess, D. 2021. Standard Operating Procedure EAP043, Version 1.4: Marine Macrobenthic Sample Analysis. Washington State Department of Ecology, Olympia. <https://apps.ecology.wa.gov/publications/SummaryPages/2303232.html>.

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Standard Operating Procedures for Marine Macrobenthic Sample Analysis

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RECERTIFIED: September 20, 2021

Please note that the Washington State Department of Ecology's Standard Operating Procedures (SOPs) are adapted from published methods, or developed by in-house technical and administrative experts. Their primary purpose is for internal Ecology use, although sampling and administrative SOPs may have a wider utility. Our SOPs do not supplant official published methods. Distribution of these SOPs does not constitute an endorsement of a particular procedure or method.

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

SOP Revision History

Revision Date	Rev number	Summary of changes	Sections	Reviser(s)
3/14/12	1.0	Recertified	All	Bill Kammin
1/28/15	1.1	Updated	6.1.3	Sandra Weakland, Valerie Partridge
10/6/16	1.2	Updated	9.3.1	Dany Burgess
11/1/18	1.3	Updated taxonomy procedure	9.5	Dany Burgess
3/22/2019	1.3	Formatting and accessibility updates.	All	Ruth Froese
6/30/2021	1.4	Added biomass lab procedures	1.1, 10.0	Dany Burgess

1.0 Purpose and Scope

- 1.1 This document is the Environmental Assessment Program (EAP) Standard Operating Procedure (SOP) for laboratory analysis of sediment-dwelling invertebrate samples. This includes rescreening, sorting, primary and secondary taxonomic identification, methods for obtaining size measurements and wet weights of macrofaunal organisms, and establishment of a size class reference collection for sediment-dwelling invertebrates collected from Puget Sound. This SOP covers post-collection sample processing, laboratory procedures, equipment and supplies, QA/QC procedures, safety, and data quality and reporting requirements. In general, methods will follow those described in PSEP (1987), with the exception of biomass and size class methods, which 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 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 sediment-dwelling 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 Grab sample – surficial sediment sample obtained using a van Veen sampler that has jaws closed, no washout (sample leakage from side or bottom of grab), clear overlying water, undisturbed sediment surface, and sufficient depth of penetration into the seabed.
- 3.4 SDS – Safety Data Sheets provide both workers and emergency personnel with the proper procedures for handling or working with a particular substance. A SDS 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.
- 3.5 Rescreening – process by which the benthic macroinvertebrate sample is removed from the field fixative (10% formalin) and transferred to 70% ethanol for preservation.

- 3.6 Size class – arbitrary grouping of individuals of a species which fall into a pre-determined range of measurements and developmental characteristics.
- Small (Size Class) – Benthic macroinvertebrates that are generally immature specimens in early stages of development; too small and underdeveloped to assign to genus and/or species, unless they are distinctive taxa.
 - 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.
 - Large (Size Class) – Benthic macroinvertebrates with fully-developed adult features, typically the largest specimens encountered (with the exception of x-large and megafauna); weigh ≤ 2 g.
 - X-Large (Size Class) - Benthic macroinvertebrates with fully-developed adult features, used when specimens significantly exceed the established large size class maximum but weigh ≤ 2 g.
 - Megafauna (Size Class) – Benthic macroinvertebrate weighing more than 2 g.
- 3.7 Wet weight – Refers to the weight of animal tissue including its contained water.

4.0 Personnel Qualifications/Responsibilities

- 4.1 All staff must comply with the requirements of the [EAP Safety Manual](#) (EAP, 2019). 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 their supervisor.
- 4.3.2 Read the Safety Data Sheets (SDS) for formalin, ethanol, glycerol, rose Bengal, methylene blue, methyl green, and crystal violet, before beginning the sorting/taxonomic procedures. These SDS are available in the Ecology Headquarters benthic laboratory and on the [EAP SharePoint site](#).

5.0 Equipment, Reagents, and Supplies

- 5.1 Rescreening Equipment and Supplies

- Eyewash/safety shower
- Eyewear, gloves, and footwear
- Formaldehyde monitoring badges
- Formaldehyde spill cleanup kit
- 3M spill absorbent pads
- Spill absorbent rags
- Carboy for storage of waste diluted formalin (empty ethanol carboys work well)
- Ethanol (70%, aqueous)
- 6-gallon ethanol carboy
- Rose Bengal (in a 70% ethanol aqueous solution)
- Liqui-Nox® / Alconox®
- Large Nalgene® funnel
- Sample containers
- Internal labels (Rite-in-the-Rain® paper)
- External adhesive labels
- Squirt bottles (for 70% ethanol solution and tap water)
- Electrical tape
- Clear tape
- 0.5-mm mesh sieve
- Plastic garbage bags
- Hoses, adapter, and nozzle for sink faucet
- Shallow plastic basins or trays
- Spoons
- Forceps
- HB graphite pencils
- Pens

5.2 Sorting Equipment and Supplies

- Eyewash/safety shower
- 0.5mm mesh sieve
- Vials and Polyseal® caps
- Shallow plastic basins
- Spoons
- Forceps
- Vials
- Sample jars
- Labels (Rite-in-the-Rain® paper)
- Squirt bottles
- Electrical tape
- HB graphite pencils
- Pens

- Protective eyewear
- Protective gloves
- Stereo dissection and compound microscopes (magnification up to 1,000x)
- Microscope light source
- Small scalpels
- Scissors
- Gridded Petri dishes
- Glycerol
- Labeled, 6-gallon Nalgene® ethanol carboy with spigot, marked with fill lines for preparing 70% solution
- Ethanol (70%, aqueous)
- Liqui-Nox® / Alconox®
- Calcium carbonate
- Rose Bengal (in a 70% ethanol aqueous solution)

5.3 Taxonomic Identification and Biomass/Size Class Equipment

- Stereo dissection and compound microscopes (magnification up to 1,000x)
- Microscope light source
- Cover slips
- Labels (Rite-in-the-Rain® paper)
- Vials and Polyseal® caps
- Small scalpels
- Scissors
- Vials
- Cotton
- Forceps
- Petri dishes
- Glycerol
- Ethanol (70% aqueous), in squirt bottles
- Methylene blue
- Methyl green
- Crystal violet
- Clear plastic ruler
- Clear tape
- Flexible Measuring Tape
- Laboratory scale with 0.0001 g resolution

5.4 **Permits**

- 5.4.1 Apply for Washington State ethanol permit by end of May (permits are required to purchase ethanol).

A Class 2 Special Permit from the Washington State Liquor and Cannabis Board must be obtained each year. The permit period is July 1 of the current year to June 30 of the following year. The permit application must be submitted and received by June 30 each year. For state agencies, there is no cost for the permit. The application form (LIQ 354-50) is currently available at:

<http://www.liq.wa.gov/publications/Liq35450class126app.pdf>. More information is given in a brochure currently available at:

<http://www.liq.wa.gov/publications/IndAlcoholinfosheet.pdf>.

5.4.2 A Federal alcohol permit must have already been obtained from the U.S. Department of the Treasury – Alcohol and Tobacco Tax and Trade Bureau.

The Environmental Assessment Program has a federal Industrial Alcohol User Permit, permit number TF-WA-470, obtained in 1990 and last amended in 2016. The permit does not need to be renewed unless there are changes in signing authority (who can buy and use ethanol). To edit signing authority, a new Signing Authority Form (TTB F 5100.1) must be submitted along with a cover letter from the Program Manager and an organization chart. Currently, the signing authority covers the users of ethanol within EAP (personnel who are using ethanol to preserve biological specimens) and their management up to Program level. The Signing Authority Form may be obtained at <https://www.ttb.gov/images/pdfs/forms/f51001.pdf>.

5.5 Throughout the year, fix any equipment that needs repairs, and order and assemble all supplies (above) needed.

5.6 Prepare all labels:

5.6.1 Whole sample labels must include project name, sample number, collection date, and sieve size.

5.6.2 Major phylum sorting labels must include project name, sample number, collection date, and phylum.

5.6.3 Voucher labels must include name, sample number, collection date, species name, and taxonomist name.

5.7 Prepare rescreening schedule.

5.8 Reserve hood in the Marine Waters laboratory.

6.0 Summary of Procedure

6.1 **Field Preservation of Samples:** Immediately following field collection, samples will be fixed in a 10% aqueous solution of borax-buffered formalin (see Shipboard Sediment Protocol for further explanation). They will remain in this solution for a minimum of 24 hours and a maximum of 7 days to allow proper fixation of the animal tissue while minimizing loss of calcium carbonate structures (e.g., mollusk shells, echinoderm spicules).

- 6.2 All sample-processing activities (including rescreening, sorting, and sorting quality control) will be recorded on a Sample Tracking Log.
- 6.3 **Rescreening Infaunal Samples Preserved with Formalin** – Rescreening is the process by which sample material is removed from the field fixative (10% formalin) and transferred to 70% ethanol for preservation. Rescreening is conducted indoors, inside a full-sized fume hood in a properly ventilated closed room. Steps in the rescreening procedure include the following:
- 6.3.1 Post warning notice on the door to the fume hood room indicating that formaldehyde is in use and that there is risk of exposure to airborne formaldehyde.
- 6.3.2 Have the fume hood fan running at all times.
- 6.3.3 Keep spill absorbent rags and Formaldehyde Spill Kit accessible throughout the procedure.
- 6.3.4 Place the funnel in the opening of the waste formalin container. Set the sieve in the funnel. Select a sample and open it inside the fume hood. Screw appropriately sized 0.5-mm meshed lid onto sample container. Slowly pour the liquid into the waste formalin container. Rinse the sample container with clean water and pour through sieve into the waste formalin container. Rinse several times into the hood sink to remove residual formalin.
- 6.4 Fill the jar up to the top with 70% ethanol, leaving approximately ½-inch headroom. Invert the jar several times to ensure even distribution of the preservative.
- 6.4.1 Stain 1.0-mm sample fraction with one generous squirt of Rose Bengal solution per sample.
- 6.4.2 Monitor the five-gallon waste formalin container, making sure the container is not overfull.
- 6.5 **Sample Tracking:** After the sample has been rescreened, the jar must be checked into the Sample Tracking Log.

- 6.5.1 After samples have been checked into the Sample Tracking Log, they will be placed in Flammable Storage Cabinets located in well-ventilated, secured storage rooms until sorting has begun. Multiple jars of a sample will be labeled “1 of 2,” “2 of 2,” etc., and stored together.
- 6.5 **Sample Sorting:** Sorting is the process of removing all faunal material from the sediment sample. The sorting process is accomplished as follows:
- 6.5.1 Each sample should be sorted by only one person. The sorter will select a station and remove all jars for that station from the storage area.
- 6.5.2 The name of the sorter and date will be recorded on the Sample Tracking Log beside the appropriate sample number.
- 6.5.3 The sorter will select five vials or jars, one for each of the following taxonomic groups: Annelida, Arthropoda, Mollusca, Echinodermata, and Miscellaneous Phyla. Each vial will receive an internal label with the survey name, station name, collection date, field screen size, and taxonomic category. 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. Each vial or jar will be filled with 70% ethanol and tightly sealed with a Polyseal cap.
- 6.5.4 Immediately prior to sorting, the ethanol will be decanted from the sample using a sieve with a mesh size one-half that of the original field mesh size and the sample will be gently rinsed with fresh water and placed in fresh water for the duration of the workday. The decanted ethanol will be retained for re-preserving the sample residue after sorting is completed. If sorting takes more than one or two days, the sample should be replaced in ethanol overnight to prevent decomposition.
- 6.5.5 Sorting will be performed using a dissecting light microscope with a minimum capacity of 10-power magnification.
- 6.5.6 A small amount of the sample (e.g., teaspoonful) will be placed in a gridded Petri dish and suspended in fresh water.
- 6.5.7 The sediment will be examined systematically using the microscope. All whole macroinfaunal invertebrates and fragments of organisms that were alive at the time of preservation are to be removed from the sample and sorted into the five phyla groups (Annelida, Arthropoda, Mollusca, Echinodermata, and Miscellaneous Phyla). Experienced sorters should count each animal as it is removed; new sorters and volunteers do not need to do counts. Meiofaunal organisms such as nematodes and foraminiferans do not need to be removed from the sample. Colonial organisms such as hydrozoans, sponges, and bryozoans will be removed completely from the sample. This includes all colony fragments and all parts of colonies attached to hard surfaces such as worm tubes, shells, or rocks (the substrata may be included in the vial with the organisms). Colonials do not need to be counted during sorting.

6.5.8 The sorter should examine each dish of sediment at least twice, swirling the dish gently between examinations, until one complete examination yields no organisms.

6.5.9 To make sorting easier, samples may be fractionated by one of two methods:

6.6.9.1 The graded sieve method uses a set of nested sieves of diminishing mesh sizes to separate the sediment by particle size. The sample is washed through the nested sieves with a gentle stream of water to prevent loss or damage to organisms.

6.6.9.2 The elutriation method is used for samples with a large amount of sand or gravel. Small portions of the sample are placed in a beaker with water and swirled to suspend the lighter fractions, which are then quickly decanted into a second beaker or sieve. This process is repeated until little or no suspended material is left. Both fractions are sorted. Most of the organisms in the sample will be concentrated in the lighter fraction.

6.7 **Sample Tracking:**

6.7.1 The following will be recorded on the Infaunal Sample Tracking Log once sorting of a sample is completed: number of animals in each phyla group, number and category of vials generated for each sample, and the sorting time (in hours).

6.7.2 The sorted residue will be returned to the original sample jars and preserved with the reserved ethanol. The sample will be recorded on the Infaunal Sample Tracking Log as sorted and the residue returned to the storage area.

6.8 **Taxonomic Identification of Organisms:**

6.8.1 Identification and enumeration of sorted organisms will be performed to the lowest taxonomic level possible, usually to species. The identifications will be done by in-house taxonomists, using minimum 10x magnification dissecting light microscopes and compound light microscopes equipped with 10x, 20x, 40x, 63x, and 100x magnification objective lenses.

6.8.2 If an organism cannot be identified to species level, the taxonomist must decide whether 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):

6.8.2.1 Exclude a taxon if ALL of the following are TRUE:

- The ID is not at species level (e.g., family or genus).
 - 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).

- 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*.)

6.8.2.2 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.

6.8.2.3 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.

6.8.3 Taxa designated as needing QA/QC will go into separate cryoseal vials with 70% ethanol and 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. Specimens not needing QA/QC will go back into the original vials containing the sorted phyla groups.

6.8.4 Taxonomists will maintain a collection of notes and comments on the organisms in each sample including descriptions of any new, exotic, or unusual species. Notes made by in-house taxonomists and copies of notes from contract taxonomists will be kept in the laboratory.

6.8.5 Taxonomists will be required to provide a verified voucher collection of all taxa encountered during the various monitoring programs, used as a reference for verifying identifications. Each vial in the collection will contain specimens from a single station. Ecology personnel will maintain all voucher collections in the Ecology benthic laboratory and add to the collection regularly.

6.8.6 **Biomass and size class of organisms:** In addition to taxonomic identifications, in-house taxonomists will be responsible for assigning each organism to one of five size categories, each associated with a corresponding biomass estimate. Biomass and size class techniques are 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 those studies include three size class designations, but methods have been revised to include a 4th category for megafauna (Tara Macdonald, personal communication). The methods outlined below reflect this change.

6.8.6.1 **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 five size classes (small, medium, large, x-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):** The smallest individuals of a taxon; 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.
- **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 2/3 the size of those classified as large (see below).
- **Large (L):** Typically the largest specimens encountered (with the exception of x-large and megafauna); weigh ≤ 2 g
- **X-Large (XL):** Used when specimens significantly exceed the established large size class maximum but weigh ≤ 2 g.
- **Megafauna (M):** All specimens weighing >2 g.

6.8.6.2 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 (this information is recorded in a spreadsheet along with the size class cutpoints for each taxon). 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.

6.8.6.3 Obtain a corresponding wet weight for each individual animal measured in section 6.7.7.2. Wet weights are performed with a balance that is accurate to 0.0001 g and that has been properly calibrated and leveled (using the leveling bubble near the front display). 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. If taxa are below the balance’s detection limits and read as 0.0000 g, these are reported as 0.0001 g.

- 6.8.6.4 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.
- 6.8.6.5 Epifaunal organisms such as: barnacles, colonial hydroids, bryozoans, sponges, colonial tunicates, etc. should be weighed in bulk for each sample, and the bulk weight recorded in a separate biomass table in the database. Solitary members of these groups, including solitary tunicates and anemones, may be individually measured, weighed, and assigned a size class.
- 6.8.6.6 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. For taxa that contain a large amount of liquid (sea cucumbers, large polychaetes, etc), specimens should be punctured and drained before weighing.
- 6.8.6.7 **Calculation of megafaunal live/dead correction factor:** Live/field IDed animals weigh more than preserved ones because ethanol has a dehydrating effect on tissues, and a correction factor (CF) must be calculated for each megafaunal taxon to account for loss of body weight during preservation.

In order to develop the CF for a given taxon, specimens of that taxon will be weighed in both the field and lab, and the following calculated:

$$\text{lab (preserved) weight} \div \text{field (live) weight} = \text{adjusted body weight percentage}$$

The adjusted body weight percentages for all specimens of that taxon are then averaged to get the CF:

$$\text{sum of adjusted body weight percentages} \div \text{number of specimens weighed} = \text{CF}$$

The taxon-specific CF can then be used to estimate preserved weight from a field-weighed specimen:

$$\text{CF} \times \text{field weight} = \text{preserved weight (corrected)}$$

This information should be entered into the megafaunal biomass database and used to determine whether megafaunal taxa qualify for field workup and release.

- 6.8.6.8 **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 one 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).

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

- 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 are few 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.
- Larval organisms (e.g. crab megalopae, zoea, or hydromedusae)
- Incidental pelagic organisms such as copepods

6.8.6.10 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 x-large class is intended to capture anything falling outside the range of the largest size class, but smaller than megafauna, so the cutpoint between x-large and megafauna is determined by weight rather than length. 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 so 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 6.6.2, assign each organism entered in the Taxon Size Classes table to one of the five 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.

6.8.6.11 Calculate the mean (arithmetic average) wet weight for each of the five 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.

6.8.6.12 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 (individual organisms will not need to be weighed). These rules apply only to the small, medium, large, and x-large size classes - megafauna should always be weighed and measured individually due to the wide variability in the size of these organisms.

6.8.6.13 The reference collection should be updated/added to following each subsequent sampling event. Specimens falling into any of the following categories should be weighed and measured as described above:

- New taxa with no data previously recorded
- Existing, rare taxa for which there are few data
- Existing taxa for which there may be plenty of data, but the length/weight of the specimen in question falls outside the range of established size classes for that taxon.

6.8.7 **Disposition of Identified Samples:** Upon completion of all identifications, biomass, and quality control procedures, the sample specimen vials will be topped off with 70% ethanol and the Polyseal caps tightly sealed. Larger containers such as jars will be wrapped with Parafilm around the vial neck to inhibit evaporation. Samples will be grouped by phyla, placed into tightly covered plastic basins, and placed in a secure, well-ventilated, cool storage room. Samples will be checked periodically for excessive evaporation of preservative and refilled when necessary.

6.8.8 **Disposal timeline:** In order to conserve storage space, it may be necessary to periodically dispose of archived samples once the data have been QAed and published. Archived samples may be discarded after five years from the date of collection for long-term samples, and six years after the date of collection for Urban Bays samples.

7.0 Records Management

- 7.1 Chain of custody procedures will follow those recommended by the PSEP (1996b). They will be initiated when the first sample is collected and will be followed until all samples are relinquished to the analytical laboratory. Chemistry, bioassay, and infaunal chain of custody forms designed for this project will provide an unbroken trail of accountability that ensures the physical security of samples, data, and records. At the end of each sample processing day, all sample containers are checked against chain of custody forms. It is important to verify the station identification number, collection date, and, if applicable, taxon as part of the QA/QC procedures.
- 7.2 Laboratory measurements, weights, size class, and comments will be entered directly into a database: currently <F:\EAP\MSMP\Infaunal sample tracking\PSEMP Taxonomy data entry.accdb>. All data submissions must meet the following formatting requirements:
- Date – collection date
 - Species or taxon name if animal could not be identified to species. It is essential that all taxon names be standardized in spelling and form. The taxon name should be free of all forms of punctuation.
 - Qualifier – any qualifier describing the result
 - Abundance – number of individuals
 - Exclude – whether the result should be excluded from statistical analyses
 - Voucher – number of individuals transferred to the voucher collection
 - Comments – any comments associated with the result
 - Whether QA/QC is needed for a taxon, and name of secondary taxonomist

8.0 Quality Control and Quality Assurance

8.1 Sorting Quality Control

- 8.1.1 To determine sorting efficiency and ensure that all organisms are removed from the sediment, a quality control check will be completed for every sample sorted. If the sorter is new or a volunteer, one-hundred percent of each sample will be re-examined by an independent sorter. For experienced sorters, twenty-five to one-hundred percent of each sample will be re-examined by an independent sorter to determine whether a sorting efficiency of 95% removal of organisms is achieved. Using best professional judgment, the quality control technician has the option to completely re-sort small or difficult-to-divide samples, while large samples can be subdivided, with no less than one quarter of the sample being re-examined.
- 8.1.2 All organisms found in the sample during the quality control check are counted, identified to major taxa group, and placed in the appropriate major taxa vial for that sample. The sample will have passed the quality control check if the number (or estimated number) of organisms found during the resort does not differ from the original count by greater than five percent. If the sample fails, then the entire sample must be resorted. For new sorters or volunteers, “training” should be marked in the pass/fail column of the quality control spreadsheet.

8.2 Taxonomic Quality Control

- 8.3 Consistency of the work of primary taxonomists for each taxa group will be achieved by various taxonomic standardization methods including the production of voucher sheets with descriptions of all taxa (verified by regional experts) and by continual interaction between primary and secondary taxonomists including taxonomic workshops. Quality control for primary taxonomists will be provided by the re-identification of 5% of bulk samples and any “problem taxa” by a chosen secondary taxonomist.
- 8.4 The benthic macroinvertebrate database contains multiple QA/QC queries intended to check for missing fields, mismatched abundances and size class totals, and incorrect species names in taxonomic datasets. These queries should be run before generating chains-of-custody used to request QA/QC bids from secondary taxonomists.
- 8.5 The Puget Sound Benthos Size Class Reference Collection (see section 6.7.7.1) 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 Chemical Safety Data Sheets (SDSs) for all chemicals used in the procedures outlined in this SOP can be found on the [EAP SharePoint site](#). Also, binders containing SDSs can be found in all field vehicles, vessels, Ecology buildings, or other locations where potentially hazardous chemicals may be handled. EAP staff that follow Ecology SOPs are required to familiarize themselves with these SDSs and take the appropriate safety measures for these chemicals.
- 9.2 The SDS for the following chemicals can be found on the EAP SharePoint site:
- Ethanol (100% and 70% aqueous solution)
 - Glycerol
 - Rose Bengal
 - Methylene Blue
 - Methyl Green
 - Crystal Violet
 - Formaldehyde and Formalin

9.3 Employee’s Responsibilities

- 9.3.1 Read this standard operating procedure and discuss any questions with supervisor or task team leader.

- 9.3.2 Read all Safety Data Sheets (SDS) before beginning this procedure. The SDS are available in the Ecology benthic laboratory or at the web links above.
- 9.3.3 Participate in Ecology Formalin Safety Training.
- 9.3.4 Report to supervisor immediately any symptoms or reactions that might be related to formalin exposure.
- 9.3.5 Properly use protective clothing and equipment.
- 9.3.6 Refrain from wearing contact lenses during this procedure.
- 9.3.7 Immediately flush with water any skin area that comes into contact with formalin.

9.4 **Protective Clothing and Equipment**

- 9.4.1 Safety splash goggles.
- 9.4.2 Chemical resistant gloves.
- 9.4.3 Chemical resistant apron.
- 9.4.4 Formalin monitoring badges.
- 9.4.5 Portable Eyewash/Safety station.
- 9.4.6 Formalin spill cleanup kits.

9.5 **Monitoring**

- 9.5.1 Representative monitoring for airborne formalin shall be conducted at the inception of a new procedure involving handling of formalin or at the resumption of an established procedure after a long period of time has elapsed.
- 9.5.2 The exposure of each employee involved in sample processing will be determined, using appropriate short-term exposure or long-term exposure monitoring badges.
- 9.5.3 Complete records of the results of airborne formalin monitoring will be kept.

9.6 **Clean up**

- 9.6.1 If a large formalin spill occurs on the vessel, use an absorbent material to soak up the majority of the free liquid. Then rinse the area thoroughly with copious amounts of water. Scrub the area with Liqui-Nox and water if it is a large spill.
- 9.6.2 If formaldehyde or formalin has contaminated your clothing, you should change into clean clothing immediately.
- 9.6.3 Do not take contaminated work clothes home. An authorized individual should launder contaminated work clothes.
- 9.6.4 All protective clothing should be thoroughly scrubbed with Liqui-Nox and water.
- 9.6.5 If personal exposure to formaldehyde or formalin occurs, wash all body areas IMMEDIATELY and THOROUGHLY, with copious amounts of water.
- 9.7 **Hazardous waste disposal**
- 9.7.1 The Washington State Department of Ecology has a Hazardous Waste Disposal Contractor. This contractor must be contacted at least one week in advance to pick up accumulated waste formalin.
- 9.7.2 Waste formalin is stored in 5-gallon drums that are required to be marked with appropriate Hazardous Materials labels, an accumulation start date label, and the name and number of the responsible employee.
- 9.7.3 Once 220 pounds have been accumulated, the waste formalin must be disposed of within 90 days. The accumulation start date must be recorded on the drum.
- 9.7.4 Five-gallon buckets of sorted contaminated sediments may be disposed of by Ecology's Hazardous Waste Disposal Contractor.
- 9.7.5 Caution must be exercised when handling samples preserved in formalin because it is a strong irritant, toxic, and carcinogenic (Kitchens et al., 1976). Copies of the SDS are kept in the benthic laboratory on a clipboard and in the EAP Safety Officer's office on the second floor of the Headquarters building.
- 9.7.6 Sorted sediment – Upon completion of all sample sorting and quality control procedures, the residual sediment from sorted samples may be disposed of by decanting 70% ethanol over a 0.5-mm screen and rinsing the sediment with copious amounts of water. Once the water has drained, the sediment will be placed into a properly labeled 5-gallon bucket with an airtight lid. When the bucket has been filled, it will be tightly sealed and properly disposed of.

9.7.7 Waste ethanol, glycerol (extremely small quantities), and vital stain mixtures (extremely small quantities) may be flushed down the drain with copious amounts of water.

10.0

References

- 10.1 Burd, B.J., T.A. Macdonald, and A. van Roodselaar. 2012a. Towards predicting basin-wide invertebrate organic biomass and production in marine sediments from a coastal sea. *Plos One* 7(7): e40295. Supplemental Material: <http://www.plosone.org/search/simple;jsessionid=84E93FDB92ED534A87B60929F34392F6?from=globalSimpleSearch&filterJournals=PLoSONE&query=Burd&x=0&y=0>.
- 10.2 Burd, B., S. Bertold, and T. Macdonald. 2012b. Responses of infaunal composition, biomass and production to discharges from a marine outfall over the past decade. *Marine Pollution Bulletin* 64(9):1837-52.
- 10.3 Burd, B., T. Macdonald, and S. Bertold. 2013. The effects of wastewater effluent and river discharge on benthic heterotrophic production, organic biomass and respiration in marine coastal sediments. *Marine Pollution Bulletin* 74, 251-263.
- 10.4 Burd, B. 2014. Distribution, inventory and turnover of benthic organic biomass in the Strait of Georgia, Canada, in relation to natural and anthropogenic inputs. *Marine Pollution Bulletin* (online) <http://dx.doi.org/10.1016/j.marpolbul.2014.03.004>
- 10.5 CORI (Vancouver Aquarium's Coastal Ocean Research Institute). 2015. Proposal to PSEMP sediment group for harmonization of benthic invertebrate and sediment conventionals monitoring with SSAMEx.
- 10.5 Dutch, M., V. Partridge, S. Weakland, K. Welch, and E. Long. 2009. Quality Assurance Project Plan: The Puget Sound Assessment and Monitoring Program Sediment Monitoring Component. Publication No. 09-03-121. Washington State Department of Ecology, Olympia.
- 10.6 Environmental Assessment Program. Environmental Assessment Program Safety Manual. March 2019. Washington State Department of Ecology, Olympia.
- 10.7 Macdonald, T., B. Burd, and A. van Roodselaar. 2012a. Facultative feeding and consistency of trophic structure in marine soft-bottom communities. *Marine Ecology Progress Series* 445, 129-140.

- 10.8 Macdonald, T., B. Burd, and A. van Roodselaar. 2012b. Size Structure of Marine Soft-Bottom Macrobenthic Communities across Natural Habitat Gradients: Implications for Productivity and Ecosystem Function. PLoS ONE 7(7), e0040071. Supplemental Material: <http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0040071>.
- 10.9 Manchester Environmental Laboratory [MEL]. 2016. Manchester Environmental Laboratory Lab Users Manual Tenth Edition. Environmental Assessment Program. Washington State Department of Ecology. Manchester.
- 10.10 Puget Sound Estuary Program [PSEP]. 1987. Recommended Protocols for Sampling and Analyzing Subtidal Benthic Macroinvertebrate Assemblages in Puget Sound: Final Report. Prepared by Tetra Tech, Inc. for U. S. Environmental Protection Agency Region 10, Office of Puget Sound.
- 10.11 Puget Sound Estuary Program [PSEP]. 1996a. Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound. Prepared by Tetra Tech, Inc. for U. S. Environmental Protection Agency Region 10, Office of Puget Sound.
- 10.12 Puget Sound Estuary Program [PSEP]. Recommended Quality Assurance and Quality Control Guidelines for the Collection of Environmental Data in Puget Sound. Prepared by Tetra Tech, Inc. for U. S. Environmental Protection Agency Region 10, Office of Puget Sound.
- 10.13 Southern California Coastal Water Research Project [SCCWRP]. 2013. Southern California Bight 2013 Regional Marine Monitoring Survey (Bight'13) - Macrobenthic (Infaunal) Sample Analysis Laboratory Manual. Prepared by the Bight'13 Benthic Committee for the Commission of the Southern California Coastal Water Research Project. Costa Mesa, CA. http://ftp.sccwrp.org/pub/download/DOCUMENTS/BightPlanningDocuments/Bight13/B13_BenthicLabManual.pdf.