Washington State Department of Ecology

Environmental Assessment Program

Standard Operating Procedures for Marine Macrobenthic Sample Analysis

Version 1.2

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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.

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 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

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, including rescreening, sorting, and primary and secondary taxonomic identification of samples of 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. Methods will, in general, follow those described in PSEP (1987).

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 Grab Sample – surficial sediment sample obtained using a vanVeen 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.

3.2 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.

3.3 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.

3.4 Re-screening – process by which the benthic macroinvertebrate sample is removed from the field fixative (10% formalin) and transferred to 70% ethanol for preservation.

4.0 Personnel Qualifications/Responsibilities

4.1 All staff must comply with the requirements of the EA Safety Manual (EA Program, 2006). 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 <u>Rescreening Equipment and Supplies</u>

5.1.1 Eyewash/safety shower
5.1.2 Eyewear, gloves and footwear
5.1.3 Formaldehyde monitoring badges
5.1.4 Formaldehyde spill cleanup kit
5.1.5 3M spill absorbent pads
5.1.6 Spill absorbent rags
5.1.7 Carboy for storage of waste diluted formalin (empty ethanol carboys work well)
5.1.8 Ethanol (70%, aqueous)
5.1.9 6-gallon Ethanol Carboy
5.1.10 Rose Bengal (in a 70% ethanol aqueous solution)
5.1.11 Liqui-Nox® / Alconox®
5.1.12 Large Nalgene® funnel
5.1.13 Sample containers
5.1.14 Internal labels (Rite-in-the-Rain® paper)
5.1.15 External Adhesive labels
5.1.16 Squirt bottles (for 70% ethanol solution and tap water)
5.1.17 Electrical Tape
5.1.18 Clear Tape

5.1.19 0.5-mm mesh sieve
5.1.20 Plastic garbage bags
5.1.21 Hoses, adapter, and nozzle for sink faucet
5.1.22 Shallow plastic basins or trays
5.1.23 Spoons
5.1.24 Forceps
5.1.25 HB graphite pencils
5.1.26 Pens

5.2 Sorting Equipment and Supplies

5.2.1 Eyewash/safety shower
5.2.2 0.5mm mesh sieve
5.2.3 Vials and Polyseal® caps
5.2.4 Shallow plastic basins
5.2.5 Spoons
5.2.6 Forceps
5.2.7 Vials
5.2.8 Sample jars
5.2.9 Labels (Rite-in-the-Rain® paper)
5.2.10 Squirt bottles
5.2.11 Electrical tape
5.2.12 HB graphite pencils
5.2.13 Pens
5.2.14 Protective eyewear
5.2.15 Protective gloves
5.2.16 Stereo dissection and compound microscopes (magnification up to 1,000x)
5.2.17 Microscope light source
5.2.18 Small scalpels
5.2.19 Scissors
5.2.20 Gridded Petri dishes
5.2.21 Glycerol
5.2.22 Labeled, 6-gallon Nalgene® ethanol carboy with spigot, marked with fill lines for preparing 70% solution
5.2.23 Ethanol (70%, aqueous)
5.2.24 Liqui-Nox® / Alconox®
5.2.25 Calcium carbonate
5.2.26 Rose Bengal (in a 70% ethanol aqueous solution)

5.3 Taxonomic Identification Equipment

5.3.1 Stereo dissection and compound microscopes (magnification up to 1,000x)
5.3.2 Microscope light source
5.3.3 Cover slips
5.3.4 Labels (Rite-in-the-Rain® paper)
5.3.5 Vials and Polyseal® caps

5.3.6 Small scalpels
5.3.7 Scissors
5.3.8 Vials
5.3.9 Cotton
5.3.10 Forceps
5.3.11 Petri dishes
5.3.12 Glycerol
5.3.13 Ethanol (70% , aqueous), in squirt bottles
5.3.14 Methylene blue
5.3.15 Methyl green
5.3.16 Crystal violet

6.0 Pre-sample Processing Preparation

6.1 Throughout the year:
6.1.1 Fix any equipment that needs repairs.
6.1.2 Order and assemble all supplies (above) needed.

6.1.3 Apply for Washington State ethanol permit by end of May (permits are required to purchase ethanol)
6.1.3.1 A Class 2 Special Permit from the Washington State Liquor Control 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: www.liq.wa.gov/publications/Liq35450class126app.pdf. More information is given in a brochure currently available at: www.liq.wa.gov/publications/IndAlcoholinfosheet.pdf.
6.1.3.2 A Federal alcohol permit must have already been obtained from the U.S. Department of the Treasury – Alcohol and Tobacco Tax and Trade Bureau.
6.1.3.2.1 The Environmental Assessment Program has a federal Industrial Alcohol User Permit, permit number TF-WA-470, obtained in 1990 and last amended in 2012. 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), along with a cover letter from the Program Manager and an organization chart, must be submitted. 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 www.ttb.gov/forms/index.shtml.

6.1.4 Prepare all labels:
6.1.4.1 Whole sample labels
6.1.4.1.1 Labels will have project name, sample number, collection date, and sieve size.
6.1.4.2 Major phylum sorting labels
6.1.4.2.1 Labels will have project name, sample number, collection date, and phylum
6.1.4.3 Voucher labels

6.1.4.3.1 Labels will have project name, sample number, collection date, species name, and taxonomist name

6.1.5 Hazardous materials (formalin) training for those needing it.

6.1.6 Prepare rescreening schedule.

6.1.7 Reserve hood in the Marine Waters laboratory.

7.0 Summary of Procedure

7.1 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).

7.2 All sample-processing activities (including rescreening, sorting, and sorting quality control) will be recorded on a Sample Tracking Log.

8.0 Hazards

8.1 Ethanol (100% and 70% aqueous solution)

8.1.1 Ethanol is a highly flammable, clear liquid with a pleasant odor, and is used for long-term 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 www.sciencelab.com/msds.php?msdsId=9923955.

8.2 Glycerol

8.2.1 Glycerol is a clear, colorless and odorless liquid used to enhance preservation of benthic invertebrates. It may be harmful is swallowed. User should avoid contact with eyes, skin, and clothing, and wash hands thoroughly after handling. The MSDS for glycerol may found at www.sciencelab.com/msds.php?msdsId=9927350.

8.3 Rose Bengal

8.3.1 Rose bengal is an odorless red to brown powder. It may be harmful if swallowed. Avoid contact with eyes, skin, and clothing. Wash thoroughly after handling. Overexposure to rose bengal may cause irritation of the skin and eyes. The MSDS for Rose Bengal may be found at www.sciencelab.com/msds.php?msdsId=9924832.

8.4 Methylene Blue

8.4.1 Methylene blue is an odorless dark green crystalline powder. It may be harmful if swallowed. Avoid contact with eyes, skin, and clothing. Wash thoroughly after handling. The MSDS for methylene blue may be found at www.sciencelab.com/msds.php?msdsId=9926051

8.5 Methyl Green

8.5.1 Methyl green is an odorless dark green crystalline powder. It may be harmful if swallowed, inhaled, or absorbed through the skin. Avoid contact with eyes, skin, and clothing. Wash thoroughly after handling. The MSDS for methyl green may be found at www.sciencelab.com/msds.php?msdsId=9927668.

8.6 Crystal Violet

8.6.1 Crystal violet is a flammable dark-green powder or greenish glistening pieces having a metallic luster with a characteristic odor. It is harmful if swallowed and causes severe burns to mucous membranes and possible severe respiratory tract, skin and eye irritation. Avoid repeated or prolonged contact to this substance. Wash thoroughly after handling. The MSDS may be found at www.sciencelab.com/msds.php?msdsId=9925790.

8.7 Formaldehyde and Formalin

8.7.1 Formaldehyde is a colorless, highly reactive gas that is composed of hydrogen, carbon, and oxygen. It combines readily with many other materials and can be dissolved in water, alcohol or ether, but not in most other organic solvents with a strong odor. Formaldehyde is usually used as formalin, a liquid solution of formaldehyde diluted to 37% with water or methanol. Formalin is a very hazardous chemical with potential acute and chronic health effects; it is harmful in the case of ingestion, skin contact (especially in the eyes) or the inhalation of vapors. Prolonged exposure may result in carcinogenic effects; serious overexposure may result in death. The MSDS may be found at www.sciencelab.com/msds.php?msdsId=9924095.

8.8 Employee's Responsibilities

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

8.8.2 Read all Material Safety Data Sheets (MSDS) before beginning this procedure. The MSDS are available in the Ecology benthic laboratory or at the web links above.

8.8.3 Participate in DOE Formalin Safety Training.

8.8.4 Report to supervisor immediately any symptoms or reactions that might be related to formalin exposure.

8.8.5 Properly use protective clothing and equipment.

8.8.6 Refrain from wearing contact lenses during this procedure.

8.8.7 Immediately flush with water any skin area that comes into contact with formalin.

8.8.8 Protective Clothing and Equipment

8.8.8.1 Safety splash goggles.

8.8.8.2 Chemical resistant gloves.

8.8.8.3 Chemical resistant apron.

8.8.8.4 Formalin monitoring badges.

8.8.8.5 Portable Eyewash/Safety station.

8.8.8.6 Formalin spill cleanup kits.

8.8.9 Monitoring

8.8.9.1 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.

8.8.9.2 The exposure of each employee involved in sample processing will be determined, using appropriate short-term exposure or long-term exposure monitoring badges.

8.8.9.3 Complete records of the results of airborne formalin monitoring will be kept.

8.8.10 Clean Up

8.8.10.1 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.

8.8.10.2 If formaldehyde or formalin has contaminated your clothing, you should change into clean clothing immediately.

8.8.10.3 Do not take contaminated work clothes home. An authorized individual should launder contaminated work clothes.

8.8.10.4 All protective clothing should be thoroughly scrubbed with Liqui-Nox and water.

8.8.10.5 If personal exposure to formaldehyde or formalin occurs, wash all body areas IMMEDIATELY and THOROUGHLY, with copious amounts of water.

8.8.11 End of Work Shift

8.8.11.1 Taking a shower at the end of your work shift is recommended.

8.8.11.2 If formaldehyde or formalin has contaminated your clothing, you should change into clean clothing immediately.

8.8.11.3 Respirators should be wiped clean with 70% ethanol and returned to storage boxes.

8.8.12 Spills and emergencies

8.8.12.1 Secure the spill area. If the spill cannot immediately be contained and collected or neutralized, call the SWRO Spills Unit for help. Keep personnel not wearing protective equipment away from areas of spills or leaks until the cleanup process is completed.

8.8.12.2 If personal exposure to formaldehyde or formalin occurs, wash all body areas IMMEDIATELY and THOROUGHLY, with copious amounts of water. Emergency showers and eyewashes are available in all locations where formaldehyde may be handled.

8.8.12.3 For large spills and fires, immediately call the fire department (911).

8.8.13 Hazardous Waste Disposal

8.8.13.1 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.

8.8.13.2 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.

8.8.13.3 Once 220 pounds has been accumulated, the waste formalin must be disposed of within 90 days. The accumulation start date must be recorded on the drum.

8.8.13.4 5-gallon buckets of sorted contaminated sediments may disposed of by Ecology's Hazardous Waste Disposal Contractor.

8.8.13.5 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 MSDS 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.

8.8.13.6 Sorted sediment - Upon completion of all sample sorting and quality control procedures, the residual sediment from sorted samples may be disposed of by decanting the 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.

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

9.0 Detailed Procedures

9.1 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:

9.1.1 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.

9.1.2 Have the fume hood fan running at all times.

9.1.3 Keep spill absorbent rags and Formaldehyde Spill Kit accessible throughout the procedure.

9.1.4 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.

9.1.5 Fill the jar up to the top with 70% ethanol, leaving approximately ½ inch head room.

9.1.6 Stain 1.0-mm sample fraction with one generous squirt of Rose Bengal solution per sample.

9.1.7 Monitor the five-gallon waste formalin container, making sure the container is not overfull.

9.2 Sample Tracking

9.2.1 After the sample has been rescreened, the jar must be checked into the Sample Tracking Log. Invert the jar several times to ensure even distribution of the preservative.

9.2.2 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 is begun. Multiple jars of a sample will be labeled “1 of 2”, “2 of 2”, etc., and stored together.

9.3 Sample Sorting

9.3.1 Sorting is the process of removing all faunal material from the sediment sample. 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 following taxonomic groups: Annelida, Arthropoda, Mollusca, Echinodermata, and Miscellaneous Phyla. Meiofaunal organisms such as nematodes and foraminiferans will not 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). Organisms will be stored in vials or jars containing 70% ethanol.

9.3.2 Sorting procedures are as follows:

9.3.2.1 The sorter will select a station and sieve size and remove all jars for that station and sieve from the storage area.

9.3.2.2 The name of the sorter and date will be recorded on the Sample Tracking Log beside the appropriate sample number.

9.3.2.3 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.

9.3.2.4 Sorting will be performed using a dissecting light microscope with a minimum capacity of 10-power magnification.

9.3.2.5 A small amount of the sample (e.g., teaspoonful) will be placed in a gridded Petri dish and suspended in fresh water.

9.3.2.6 The sediment will be examined systematically using the microscope, and all animals and parts of benthic animals will be removed. The sorter should examine each dish of sediment at least twice, swirling the dish gently between examinations, until one complete examination yields no organisms.

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

9.3.3.1 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.

9.3.3.2 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.

9.3.4 Each sample should be sorted by only one person. Organisms should be sorted into the major phyla; Annelida, Arthropoda, Mollusca, Echinodermata, and Miscellaneous Phyla. All organisms will be sorted into vials containing 70% ethanol and tightly sealed with Polyseal caps.

9.4 Sample tracking and labeling of sorted samples

9.4.1 Each organism vial will have 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 a HB graphite pencil.

9.4.2 The number and category of vials generated for each sample will be recorded, along with the sorting time, on the Infaunal Sample Tracking Log. 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.

9.5 Taxonomic Identification of Organisms

9.5.1 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, 40x and 90x and 100x magnification objective lenses.

9.5.2 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.

9.5.3 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

9.6 Disposition of Identified Samples

9.6.1 Upon completion of all identifications 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. All vials from each replicate sample and station will be bundled together. All samples from the survey will be 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.

10.0 Quality Control and Quality Assurance Section

10.1 Sorting Quality Control

10.1.1 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. 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 resort small or difficult-to-divide samples, while large samples can be subdivided, with no less than one quarter of the sample being re-examined.

10.1.2 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.

10.1.3 The quality control technician will also check all major taxa vials for missorted organisms (i.e., organisms placed in the incorrect vials).

10.2 Taxonomic Quality Control

10.2.1 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.

10.3 Records Management

10.3.1 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.

11.0 Data Submission

11.1 All data submissions must meet the following formatting requirements.

11.1.1 Date – collection date
11.1.2 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.
11.1.3 Qualifier – any qualifier describing the result
11.1.4 Abundance – number of individuals
11.1.5 Exclude – whether the result should be excluded from statistical analyses
11.1.6 Lab Code – lab which generated the result
11.1.7 Screen Size – mesh size used to collect the sample
11.1.8 Voucher – number of individuals transferred to the voucher collection
11.1.9 Comments – any comments associated with the result

12.0 References

12.1 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. Washington State Department of Ecology Publication 09-03-121. 98 pp.

12.2 Environmental Assessment Program, 2006. Environmental Assessment Program Safety Manual. March 2006. Washington State Department of Ecology. Olympia, WA.

12.3 MEL, 2016. Manchester Environmental Laboratory Lab Users Manual Tenth Edition. Environmental Assessment Program. Washington State Department of Ecology. Manchester, WA.

12.4 PSEP (Puget Sound Estuary Program), 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.

12.5 PSEP (Puget Sound Estuary Program), 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.

12.6 PSEP (Puget Sound Estuary Program), 1996b. 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.