Appendix. Overview

Standard Operating Procedures - West Coast Invertebrate Genomics Initiative

All procedures developed for the West Coast Invertebrate Genomics Initiative are adapted from

- *Washington State Department of Ecology Standard Operating Procedures*
- *Procedures provided by Dr. Gustav Paulay, Florida Museum of Natural History*
- *Procedures provided by Dr. Chris Meyer and Mr. Michael O'Mahoney, Smithsonian Institution Department of Invertebrate Zoology*

(Electronic Appendices A-1 through A-9).

Field Collection of Benthic Invertebrates

- **Check itinerary daily** to see which station or stations will be sampled for benthos.
- **Collect 1-2 benthos samples** daily, usually in morning. More samples can be collected for evening processing if lab work flow permits.
- **Benthos grab sampling and shipboard sieving** generally follow Weakland, 2018a (Appendix A-2). Specimens, however, are not fixed in formalin.
- **Collect sediment grab sample** from 0.1 m^2 vanVeen grab.
- **Sieve sediment grab sample** and collect benthos using multiple stacked screens of varying mesh size, i.e. 4 mm, 2 mm, 1 mm screen.

Note: Chris Meyer, Smithsonian, would like to collect some of the meiofauna that go through the 1.0 mm screen. He has asked that we collect these on a 0.5 mm screen for a few samples, if time permits. The organisms collected on the 0.5 mm screen from a station should be accumulated in one sampling container and fixed in ethanol.

- **Transfer benthos** to chemically clean plastic jars, lidded plastic trays, or plastic bags with zip closures. Place the following in separate containers:
    - Fragile animals.
    - Predators.
    - Cerianthids (try to get just the anemone…leave tube behind if possible).

    Rocks and shells will be discarded unless they have attached benthos.
- **Fill and seal** - When sample sieving is complete, partially fill each container with seawater but leave airspace on top for oxygen. Secure lid or seal, and place in chemically clean ice chest. No formalin is added to these samples. Containers can be filled to top with seawater if there will be excessive shaking during transport to lab. If this is done, then there should be less organism biomass in each container.
- **Transport ice chest** in mid- to late morning or end of sampling day to courier at designated port for transport to lab crew.

Note: If specimens do not survive from the time of sieving in the field to the time they are examined in the laboratory, then bulk sediments will be brought to the lab for sieving and processing.

- **Collect seawater in carboy(s) and transport to lab.** Keep refrigerated.

Laboratory Processing of Benthic Invertebrates for the DNA Barcode Library and Cosmopolitan Species Projects

- **Set up stations/equipment** for sample sorting, morphotyping, and taxonomic ID; microphotography; tissue collection; and voucher specimen fixation and preservation.
- **Sort sample** with chemically-clean spoon and forceps, carefully remove portions of benthos sample to clean, gridded petri dish or other sorting trays. Sort critters into 6-well plates by lots of:
    - Annelids, Arthropods, Molluscs, Echinoderms, Misc Taxa.
    - Like individuals (morphotypes).
    - Lowest possible taxon.
    - Cosmopolitan Species Project taxa: *Macoma* spp, *Ameritella* (formerly *Tellina modesta*), *Megangulus* spp. (Family Tellinidae only); Family Ampeliscidae.
- **Drain sorted residue** through fine mesh to collect debris, allowing seawater to collect in 5-gallon bucket. Dispose of seawater down sink drain, if permitted at lab, or bring back to Washington State Department of Ecology (Ecology) Benthic Lab for disposal.
- **Add appropriate relaxants** - one of magnesium chloride, menthol, clove oil, tricaine (MS-222), or cooling/freezing - to sample well as per Gustov Paulay's Specimen Processing protocols (Appendix A-7) and/or Smithsonian Institution (SI) Invertebrate Zoology Collection Procedures (Chapter 4 pg 14; Chapter 5 pg 26) (Appendix A-9).
  Note: clove oil and menthol in alcohol will remove lettering from labels. When using these relaxants, labels should not be placed in dish with organisms.
- **Process specimens**, or group of like specimens, as follows:
    - **Identify** to morphospecies (specimen lots), or lowest possible taxon (individual specimens).
    - **Label appropriately.** Hand-written field labels will accompany each sample on appropriately sturdy, archival paper stock, with #2 pencil. In the lab, a double set of pre-printed labels with unique specimen numbers are added to each individually separated specimen or specimen lot. The double labels are separated to go with each voucher and tissue sample from the same specimen, and the labels are co-photographed with each specimen, facilitating photo tracking. These specimen labels will be thermally preprinted in advance on sturdy, plastic archival stock, with lettering that does not come off.
    - **Photograph each specimen**, capturing image of the whole organism, the head, and any important taxonomic features. Use either a dissecting microscope or a macro-lens photographic set up. Photo management methods will generally follow the Paulay Photo Processing protocol (Appendix A-6).
    - **For *Cosmopolitan Species Project*:**
        - Place whole animal(s) and label in screw-top glass vial. If material exceeds 40% of the total volume of the container, use multiple containers. The specimen material:glycerin-ethanol ratio should be no greater than 1:3**.**
        - Fill vial with 95% ethanol, 5% glycerin.
        - Replace ethanol/glycerin with fresh 95% ethanol at 24 and 48 hours post-collection.
        - Store all vials in specified storage box.

- **For *Puget Sound DNA Barcode Library Project*:**
    - **Remove and preserve tissue snippet for DNA sequencing and long-term storage in SI Biorepository** – see Paulay Specimen Processing protocol (Appendix A-7) and the following from SI Collection Procedures (Chapter 5) (Appendix A-9):
        - $\leq 1cm^3$ in diameter, or between the volume of a lentil and a pea.
          Note: For most animals, there will be much less tissue collected. If a larger sample is collected, it will need to be sliced to facilitate alcohol penetration; ideally one dimension at least should not be more than 1mm thickness (G. Paulay, personal communication).
        - Harvest from a genetically dense tissue that doesn't contain degrading enzymes or taxonomically significant features.
        - Muscle tissues are the preferred sample type for invertebrates.
        - Gastric, oral, and exterior tissues are to be avoided.
        - Rinse every tissue sample with seawater.
        - Forelimb, hind limb, head, tail, mouthpart, and reproductive structures should be avoided unless otherwise specified, as these areas tend to be useful for taxonomic identification.
        - Exoskeleton and shell portions should not constitute the entirety of the sample.
        - Small size - preserve the whole specimen, and two representatives of the organism should be taken; one for the specimen voucher and one for the genetic voucher.
        - Tissue will be collected in GeneMate tissue vials with a minimum 1:10 ration of tissue to ethanol. They will be relocated to the 94-well plates at the Smithsonian.
    - **Voucher collection.** After tissue sampling, voucher specimens will be collected, fixed, preserved, and sent to both the Smithsonian Institution and the Florida Museum for long-term archiving. We will try to create duplicate collections, containing the same species.
    - **Fixative for voucher specimens**. Hard-bodied organisms (most molluscs, arthropods, and echinoderms) will be fixed in 95% ethanol, which will be replaced at 24 and 48 hours post collection with 75% ethanol. Soft-bodied organisms (worms, tunicates, cephalopods, opistobranchs) will be fixed in buffered 10% formalin, with later transfer to 75% ethanol – see Paulay Specimen Processing protocols (Appendix A-7).
    - Place each type of specimen collected in appropriate separate storage boxes.
- **Final taxonomic identification** of voucher specimens will be conducted by Ecology taxonomists when all sampling is completed, following protocols outlined in Dutch et al., 2018 and Weakland, 2018b (Appendix A-3).

Shipping

- **For the Cosmopolitan Species Project:**
    - Samples should be packaged and sent to:

      Ms. Dana Schultz (danas@sccwrp.org)

      Southern California Coastal Water Research Project Authority

      3535 Harbor Blvd

      Costa Mesa, CA 92626

      Be sure to create a tracking # for the package and e-mail that to Dana (danas@sccwrp.org), so she is expecting your delivery.

- **For Puget Sound DNA Barcode Library Project:**
    - Samples should be packaged and sent to:

      Mr. Michael O'Mahoney (OmahoneyM@si.edu)

      Department of Invertebrate Zoology

      Museum Support Center

      Smithsonian Institution

      4210 Silver Hill Rd.

      Suitland, MD 20746

- **Packaging and shipping of specimens and tissue samples in regulated fluids** (*e.g.* ethanol, formalin) will follow Ecology's Protocol for Shipping Scientific Specimens (Burgess and Eagleston, 2019; Appendix A-1) and will be in accordance with the **US Department of Transportation (DOT)** and **International Air Transport Association (IATA) regulations,** specifically **IATA Special Provision A180 (and IATA A189).**

Products and archive locations

- **Photographs of voucher specimens** – Ecology's Benthic Lab, Florida Museum of Natural History.
- **Tissue samples for DNA extraction and sequencing** – Smithsonian Institution Department of Invertebrate Zoology.
- **Tissue sample for long-term storage** - Liquid nitrogen tanks in the Smithsonian Institution Biorepository.
- **Morphological voucher specimens** – Florida Museum of Natural History, Washington State Department of Ecology, Smithsonian Institution Invertebrate Zoology, Los Angeles County Natural History Museum.

Data collection and long-term storage

- **Field** – Station information will be collected on **hand-written field logs** generated for the Puget Sound Sediment Monitoring Program as described in Dutch et al., 2018.

- **Lab –** Specimen information will be collected on **hand-written lab logs** (example in Paulay Field Template) (Appendix A-4) during sample processing, and will include information related to:
    - Specimen photographs.
    - Tissue samples for DNA extraction and sequencing, and the Smithsonian Institution Biorepository.
    - Morphological voucher collection specimens.
- **Electronic worksheets** – All station, tissue, and voucher information collected on field and lab logs will be transferred to **electronic worksheets** to be entered into relational database tables. Examples are found in:
    - SI Invertebrate Zoology Collection Info Spreadsheet (Appendix A-8).
    - Paulay Field Template (Appendix A-4).
- **Databases** – Information collected for this project will be housed in the following databases:
    - **Tissue and voucher metadata** will be archived in the Smithsonian collection's database, EMu.
    - **Barcode and specimen date** will be made public in:
        - Barcode of Life Database (BoLD) – A cloud-based data storage and analysis platform developed at the Centre for Biodiversity Genomics in Canada for a public collection of reference "Barcode" sequences from vouchered specimens of all species of life. A Barcode sequence is a short nucleotide sequence from a standard genetic locus for use in species identification.
        - GenBank® – The National Institution of Health genetic sequence database, an annotated collection of all publicly available DNA sequences.
        - The Genomic Observatories Meta-Database (GeOMe) – A web-based database which captures metadata on biological samples.
        - Symbiota – An open source *content management system* for curating specimen- and observation-based biodiversity data.
        - Integrated Digitized Biocollection (IDigbio) – A national resource for digitized information about vouchered natural history collections supported through funds from the National Science Foundation.
        - Florida Museum of Natural History – Invertebrate Zoology Collection – Records, images, and location maps for the Florida Museum's Natural History collection of invertebrates.

Quality Control and Quality Assurance

- **Field log and database entries** will all be double checked by Ecology staff and/or Dr. Gustav Paulay.
- **Taxonomic ID verification** as per 2018 Puget Sound Sediment Monitoring Program QAMP (Dutch et al., 2018; Weakland, 2018b (Appendix A-3))

Safety

- **Formalin and ethanol** – for handling, see Weakland, 2018a,b (Appendix A-2, A-3)
    - All containers filled with formalin or ethanol will be housed in separate 5-gallon over-pack pails with sorbent padding and threaded lid with a rubber O-ring seal.
    - Formalin-neutralizing pellets will be available in the event of a spill.
    - Safety goggles and chemical-resistant gloves will be used when handling chemicals.
    - Chemicals will be stored and handled in a well-ventilated area or under a fume hood.
    - All residual formalin and ethanol generated from transfer of samples from fixative to preservative will be collected and disposed of at Ecology Headquarters in Lacey following appropriate protocols.
- **MSDS sheets** will be available for formalin, ethanol, and all of the relaxants used during the study.

References

Dutch M., V. Partridge, S. Weakland, D. Burgess, and A. Eagleston. Quality Assurance Monitoring Plan: The Puget Sound Sediment Monitoring Program. Washington State Department of Ecology Publication No. 18-03-109. https://fortress.wa.gov/ecy/publications/SummaryPages/1803109.html. 127pp+appendices.

The following are housed in the electronic Appendices A-1 through A-9:

Ecology protocols

A-1. Burgess, D. and A. Eagleston. 2019. Protocol for Shipping Scientific Specimens.docx

A-2. Weakland, S. 2018a. Standard Operating Procedures for Obtaining Marine Sediment Samples EAP039 v1.3.

A-3. Weakland, S. 2018b. Standard Operating Procedures for Marine Macrobenthic Sample Analysis. EAP043 v1.2.

Dr. Gustov Paulay's protocols

A-4. Paulay Field Template.xlsx

A-5. Paulay Field Methods.docx

A-6. Paulay Photo Processing.docx

A-7. Paulay Specimen Processing.docx

Smithsonian Institution protocols

A-8. SI Invertebrate Zoology Collection Info Spreadsheet.xlsx

A-9. SI Invertebrate Zoology Collection Procedures.pdf