

**2011 Addendum to Quality Assurance Project Plan** 

The Puget Sound Assessment and Monitoring Program: Sediment Monitoring Component

January 2012 Publication No. 09-03-121-Addendum2

## **Publication Information**

### Addendum

This addendum is an annual addition and update to an original Quality Assurance Project Plan. The addendum is not a correction (errata) to the original plan.

This addendum is available on the Department of Ecology's website at www.ecy.wa.gov/biblio/0903121Addendum2.html.

#### **Original Publication**

Quality Assurance Project Plan: The Puget Sound Assessment and Monitoring Program: Sediment Monitoring Component

Publication No. 09-03-121.

The Quality Assurance Project Plan is available on the Department of Ecology's website at <u>www.ecy.wa.gov/biblio/0903121.html</u>.

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## **DEPARTMENT OF ECOLOGY**

Environmental Assessment Program

January 3, 2012

| TO:      | Puget Sound Assessment and Monitoring Program Sediment Component<br>E-mail List   |
|----------|---|
| THROUGH: | Robert F. Cusimano, Section Manager, Environmental Assessment Program<br>Carol Maloy, Unit Supervisor, Environmental Assessment Program |
| FROM:    | Margaret Dutch, Environmental Assessment Program  |
| SUBJECT: | 2011 Addendum to Quality Assurance Project Plan for: The Puget Sound Assessment and Monitoring Program: Sediment Monitoring Component   |
|          | Project Code: Project Tracker (99-510); Activity Tracker (01-900)<br>Publication No: 09-03-121-Addendum2                                |

The Washington State Department of Ecology's Marine Sediment Monitoring Team (MSMT) conducted sediment sampling in April and June, 2011, as part of their annual Puget Sound Assessment and Monitoring Program (PSAMP) and Ecology's Urban Water's Initiative (UWI) Monitoring Program. The goal of these programs is to characterize sediment quality in various regions and urban bays throughout Puget Sound.

April sampling was conducted at 10 PSAMP Long-term/Temporal monitoring stations located throughout Puget Sound. June's PSAMP Spatial/Temporal Monitoring Program sampling was conducted in the South Puget Sound sediment monitoring region. Intensive sampling occurred in Budd Inlet as part of the UWI program. Additional samples were also collected in June to measure the following:

- Nitrogen and organophosphorus pesticide, pyrethroid, and herbicide concentrations from all PSAMP and UWI stations.
- Concentrations of Pharmaceuticals and Personal Care Products (PPCP) and Perfluorinated Chemicals (PFC) from all PSAMP and UWI stations to be analyzed at the University of Washington-Tacoma (UW-T).
- Dioxin and furan concentrations in selected stations from Budd Inlet and Oakland Bay sediments as special projects for Ecology's Toxic Cleanup Program.
- Benthic invertebrates at selected stations for DNA barcoding to be analyzed at the Canadian Centre for DNA Barcoding, University of Guelph, Canada.

This addendum to the 2009 PSAMP Sediment Monitoring Component Quality Assurance Project Plan (Dutch et. al, 2009) provides details regarding all sampling locations, parameters sampled, and sample processing and quality assurance. Detailed schedules are also presented for all projects, with the exception of the PPCP/PFC and DNA Barcoding projects. Timing of completion of these projects has not yet been determined.

Additionally, the post-cruise navigation reports, including the target and actual latitude and longitude for each station and grab sample taken in April and June, are attached as appendices.

All data, data summaries, and reports generated from the PSAMP, UWI, and DNA barcoding projects will be posted to the MSMT website (www.ecy.wa.gov/programs/eap/psamp/index.htm) and Ecology's EIM database (www.ecy.wa.gov/eim/). Any questions regarding this work can be directed to Margaret Dutch at margaret.dutch@ecy.wa.gov or 360-407-6021. The dioxin and furan data will be posted to the EIM database. Questions regarding this project can be directed to Tom Gries at tgri461@ecy.wa.gov. Questions regarding the PPCP and PFC analyses can be directed to Dr. Joel Baker at jebaker@u.washington.edu and Dr. Joyce Dinglasan-Panlilio at jdingpan@u.washington.edu, UW-T.

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# **Ongoing Monitoring Programs**

## April 2011 - Ecology-PSAMP Long-Term/Temporal Monitoring

**Purpose:** To continue monitoring benthic invertebrate community structure and associated sediment quality at 10 sentinel monitoring stations representing a variety of habitat types located throughout Puget Sound.

**Sampling Details:** As described in the 2009 Quality Assurance Project Plan for the PSAMP Long-Term Temporal Monitoring Program (Dutch et al., 2009).

Station Locations: 10 historical PSAMP stations throughout Puget Sound (Figure 1, Table 1).

**Parameters Sampled:** Field measurements, macroinvertebrate abundance, grain size, total organic carbon (Table 2).

**Project Schedule:** Outlined in Table 3.

Post-cruise Navigation Report: See Appendix A.

Link to further information about this long-term program: www.ecy.wa.gov/programs/eap/psamp/TemporalMonitoring/Temporal.htm.

## June 2011 – Spatial Sediment Monitoring in South Puget Sound

Sediment sampling in June 2011 was conducted for two on-going ambient monitoring efforts, including Ecology's PSAMP Spatial/Temporal Monitoring Program and UWI. A total of 73 stations were sampled for these two projects.

The South Puget Sound regional and Budd Inlet urban bay sampling frames developed respectively for these two projects overlap, allowing sharing of some samples between the two sampling frames and collection of an equivalent number of new samples at additional locations.

Details for the PSAMP Spatial/Temporal and UWI projects are given below.

## PSAMP Spatial/Temporal Monitoring Program

**Purpose:** To characterize sediment quality in the PSAMP South Puget Sound sediment monitoring region, and determine change over time.

**Sampling Details:** As described in the 2009 Quality Assurance Project Plan for the PSAMP Spatial/Temporal and UWI Monitoring Programs (Dutch et al., 2009).

**Station Locations:** 55 randomly selected locations in the MSMT's South Puget Sound Sediment Monitoring Region, including Budd Inlet (Figure 2, Table 4). Station allocation was as follows:

- 28 Newly selected from the PSAMP Spatial/Temporal Monitoring Program sample design. All are outside of Budd Inlet.
- 12 Newly selected from the PSAMP Spatial/Temporal Monitoring Program sample design and shared with the 2011 Urban Waters Initiative Program. All are within Budd Inlet.
- 15 Originally sampled during the 1999 PSAMP/NOAA monitoring program were resampled to facilitate comparison of sediment quality conditions over time. All are outside of Budd Inlet.

A total of three stations originally targeted were rejected during sampling due to the presence of coarse gravel and rocks in the sediments that could not be sampled with the vanVeen grab. These stations were replaced with the following alternate locations:

| Target Stations<br>Rejected | Alternate<br>Station |
|-----------------------------|----------------------|
| PS00014                     | PS00164              |
| PS00260                     | PS00348              |
| PS00270                     | PS00388              |

**Parameters Sampled:** Field measurements, toxicity, macroinvertebrate abundance, grain size, total organic carbon, metals, and organic chemical contaminants (Table 5).

**Project Schedule:** Outlined in Table 6.

## Post-cruise Navigation Report: Appendix B

Link to further information about this long-term program: www.ecy.wa.gov/programs/eap/psamp/UrbanWaters/urbanwaters.htm.

## Ecology's Urban Waters Initiative (UWI) Monitoring – Budd Inlet

**Purpose:** To characterize sediment quality in the UWI Budd Inlet sampling frame, and determine change over time.

**Sampling Details:** As described in the 2009 Quality Assurance Project Plan for the PSAMP Spatial/Temporal and UWI Monitoring Programs (Dutch et al, 2009).

**Station Locations:** 30 random locations were sampled in Budd Inlet (Figure 2, Table 4). Station allocation was as follows:

- 12 Newly selected UWI locations.
- 12 Samples shared with the 2011 PSAMP Spatial/Temporal Monitoring Program.
- 6 Originally sampled during the 1999 PSAMP/NOAA monitoring program. Were resampled to facilitate comparison of sediment quality conditions over time.

All targeted stations were successfully sampled in Budd Inlet.

**Parameters Sampled:** Field measurements, toxicity, macroinvertebrate abundance, grain size, total organic carbon, metals, and organic chemical contaminants (Table 5).

**Project Schedule:** Outlined in Table 6.

Post-cruise Navigation Report: Appendix B

# Special Projects – June 2011

## Concentrations of Nitrogen and Organophosphorus Pesticides, Pyrethroids, and Herbicides in Puget Sound Sediments

**Purpose:** To establish baseline data for concentrations of nitrogen and organophosphorus pesticides, pyrethroids, and herbicides in Puget Sound sediments for the PSAMP South Puget Sound sediment monitoring region and the UWI Budd Inlet sampling frame. Samples will be analyzed at Ecology's Manchester Environmental Laboratory.

**Sampling Details:** Top 2-3cm sediments collected from a 0.1m<sup>2</sup> double vanVeen grab sampler as described in the 2009 Quality Assurance Project Plan for the PSAMP Spatial/Temporal and UWI Monitoring Programs (Dutch et al., 2009).

**Station Locations:** 73 stations, as per the 2011 PSAMP Spatial/Temporal and UWI Monitoring Programs (Figure 2, Table 4).

**Parameters Sampled:** 121 nitrogen and organophosphorus pesticides, pyrethroids, and herbicides (Table 7).

Sample Volumes and Preservation for Laboratory Analysis: Outlined in Table 8.

Laboratory Analysis and Reporting Requirements: Outlined in Table 9.

Field and Laboratory Measurement Quality Objectives: Outlined in Table 10.

**Project Schedule:** as per the 2011 PSAMP Spatial/Temporal and Urban Waters Initiative Monitoring Programs (Table 6).

**Post-cruise Navigation Report:** As per the 2011 PSAMP Spatial/Temporal and Urban Waters Initiative Monitoring Programs (Appendix B).

# Concentrations of Pharmaceuticals and Personal Care Products (PPCPs), and Perfluorinated Chemicals (PFCs) in Puget Sound sediments

**Purpose:** To establish baseline data of the concentrations of PPCPs and PFCs in Puget Sound sediments for the PSAMP South Puget Sound sediment monitoring region and the UWI Budd Inlet sampling frame. Extra sediment was collected from each June 2011 sampling location and turned over to partners at the University of Washington-Tacoma (UW-T) Environmental Science department as a pilot study for analysis of these chemicals at their new laboratory. Discussions will continue to determine whether a long-term partnership can be formed between Ecology and UW-T for continued analysis of these samples collected annually for PSAMP and UWI. These data, however, cannot be used as part of the PSAMP Spatial and UWI programs until the UW-T lab receives WA State accreditation for conduct of these analyses (www.ecy.wa.gov/programs/eap/labs/lab-accreditation.html).

**Partnership:** Dr. Joel Baker and Dr. Joyce Dinglasan-Panlilio, University of Washington-Tacoma, Department of Environmental Science.

**Sampling Details:** Top 2-3cm sediments collected from a 0.1m<sup>2</sup> double vanVeen grab sampler as described in the 2009 Quality Assurance Project Plan for the PSAMP Spatial/Temporal and UWI Monitoring Programs.

**Station Locations:** 73 stations, as per PSAMP Spatial/Temporal and UWI Monitoring Programs (Figure 2, Table 4).

**Parameters Sampled:** Field measurements, 119 PPCPs, 13 PFCs (Table 11). It is likely that only a portion of these parameters will be analyzed for by the partner lab.

Sample Volumes and Preservation for Laboratory Analysis: Outlined in Table 12.

Laboratory Analysis and Reporting Requirements: Outlined in Table 13.

Field and Laboratory Measurement Quality Objectives: Outlined in Table 14.

**Project Schedule:** Samples were collected and delivered to University of Washington-Tacoma personnel at completion of sampling in June, 2011. Dr. Joel Baker (<u>jebaker@u.washington.edu</u>) and Dr. Joyce Dinglasan-Panlilio (<u>jdingpan@u.washington.edu</u>) can be contacted for details of the project schedule.

**Post-cruise Navigation Report:** as per PSAMP Spatial/Temporal and Urban Waters Initiative Monitoring Programs (Appendix B).

## Chlorinated Dioxins and Furans in Budd Inlet and Oakland Bay

#### **Purpose:**

- To measure concentrations of 17 chlorinated dioxin and furan congeners in surface sediments of Budd Inlet and Oakland Bay.
- To use results to estimate concentrations that might represent background conditions for Budd Inlet, and to assess recovery potential for contaminated sediments in Oakland Bay.

**Partnership:** Ecology's Toxic Cleanup Program project lead: Joyce Mercuri; Ecology's Environmental Assessment Program project lead: Tom Gries

#### Sampling Details:

- **Budd Inlet:** Samples of the top 0-10 cm of surface sediment were collected from a single grab collected with a 0.1m<sup>2</sup> double vanVeen from each station. Except for the 0-10 cm depth interval of the surface sediment, sampling was conducted as described in the 2009 Quality Assurance Project Plan for the PSAMP Spatial/Temporal and Urban Waters Initiative Monitoring Programs (Dutch et al., 2009).
- **Oakland Bay:** 2 samples of surface sediment (top 0-2 cm and top 2-10 cm) were collected from a 0.1m<sup>2</sup> double vanVeen grab sample taken from each station.

#### **Station Locations:**

- **Budd Inlet:** Most sampling locations were chosen to fill spatial data gaps for PCDD/F concentrations in 0-10 cm sediment in the middle and outer portions of the inlet. The locations included 24 PSAMP Spatial/Temporal and Urban Waters Initiative Monitoring Program samples, and 6 newly selected locations. The station locations are identified in Figure 3, Table 4.
- **Oakland Bay:** Sediment samples were collected from 3 PSAMP Spatial/Temporal and Urban Waters Initiative Monitoring Program stations, and 2 newly selected locations. The station locations, identified in Figure 3 and Table 4, were chosen with hopes of identifying areas where recently-deposited (overlying) material is cleaner than older (deeper) sediments.

**Parameters Sampled:** Field measurements, grain size, total organic carbon, 17 PCDD/F congeners (Table 15).

Sample Volumes and Preservation for Laboratory Analysis: Outlined in Table 16.

Laboratory Analysis and Reporting Requirements: Outlined in Tables 17 and 18.

Field and Laboratory Measurement Quality Objectives: Outlined in Table 19.

**Project Schedule:** Outlined in Table 20.

**Post-cruise Navigation Report:** Appendix B

Link to further information about toxic cleanup work in Budd Inlet and Oakland Bay:

Budd Inlet: <a href="http://www.ecy.wa.gov/programs/tcp/sites\_brochure/budd\_inlet/budd\_inlet\_hp.htm">www.ecy.wa.gov/programs/tcp/sites\_brochure/budd\_inlet/budd\_inlet\_hp.htm</a>

Oakland Bay: <a href="http://www.ecy.wa.gov/programs/tcp/sites\_brochure/oaklandBay/oaklandBay\_hp.htm">www.ecy.wa.gov/programs/tcp/sites\_brochure/oaklandBay/oaklandBay\_hp.htm</a>

## **DNA Barcoding for Marine Benthic Invertebrates**

**Purpose:** To collect and preserve marine benthic invertebrate samples for taxonomic identification and DNA barcoding analysis at the Canadian Centre for DNA Barcoding (Centre) (www.dnabarcoding.ca/, University of Guelph, Canada.

Puget Sound marine invertebrate taxa barcoding data will be added to the Barcode of Life Data System (BOLD), an online data management system which is central to the global barcoding community for maintaining barcode records and providing a resource to identify unknown animals (<u>www.boldsystems.org/views/login.php</u>).

Barcoding data will also be used by regional taxonomists in Puget Sound and Southern California to distinguish species typically grouped into "complexes" due to lack of morphologically distinct external features, and by West Coast taxonomists to determine whether species identified over wide geographic ranges (e.g., California to Puget Sound) are genetically, as well as morphologically, the same (see DNA Barcoding Project Proposal, Appendix C).

**Partnership:** Dr. Bonnie Becker, University of Washington-Tacoma (and student interns), Department of Environmental Science; Dr. Eric Stein, Southern California Coastal Water Research Project (SCCWRP); Dr. Peter Miller, Canadian Centre for DNA Barcoding; citizen volunteers from Puget Sound.

**Sampling Details:** Benthic invertebrate samples will be collected from sediment monitoring stations and sieved from the sediment matrix during the course of sampling as per established PSAMP/UWI protocols (Dutch, 2009). They will be preserved in 100% ethanol, sorted and identified to the species level, and their tissue harvested and shipped to the Centre as per developed protocols (Appendix D). Data will then be incorporated into the BOLD database and publically available.

**Station Locations:** Benthic invertebrates were collected from 12 PSAMP Spatial/Temporal and Urban Waters Initiative Monitoring Program samples. Station locations are identified in Figure 4, Table 4.

Parameters Sampled: Marine benthic invertebrates.

Sample volumes and preservation for laboratory analysis: Invertebrates were removed from  $0.1m^2$  sediment grab samples collected from one or both sides of a double vanVeen grab. Preservation methods in ETOH followed those developed by the Centre, as adapted for the PSAMP (Appendix D).

Laboratory analysis and reporting requirements: see Appendix D.

**Field and laboratory Measurement Quality Objectives:** All field and laboratory Quality Assurance/Quality Control procedures for collection, sieving, and sorting of benthic invertebrate samples will follow those in the established PSAMP/UWI project plan (Dutch et al., 2009).

**Project Schedule:** This project is currently unfunded. Collection and sieving of invertebrates in the field occurred as time permitted. Student interns from Dr. Bonnie Becker's lab at the University of Washington-Tacoma, as well as citizen volunteers, supervised by MSMT staff, will conduct the sorting of barcode samples. Regional taxonomists will be conducting species-level identification of sorted organisms as a volunteer service. Invertebrates that have been sorted and identified will then be sent to the Centre for tissue preparation and DNA barcoding. The time frame for generation of DNA barcoding reports, as outlined in Appendix C, has yet to be determined.

**Post-cruise Navigation Report:** as per PSAMP Spatial/Temporal and Urban Waters Initiative Monitoring Program (Appendix B).

# **Future Sediment Monitoring**

Future monitoring locations and sampling dates for the PSAMP and UWI programs listed above are indicated in the schedule in Table 21.

For further information or comments, contact Maggie Dutch at 360-407-6021 or <u>margaret.dutch@ecy.wa.gov</u>.

# Literature Cited

Dutch, M., V. Partridge, S. Weakland, K. Welch, E. Long. 2009. Quality Assurance Project Plan: The Puget Sound Assessment and Monitoring Program Sediment Monitoring Component. Washington State Department of Ecology Publication No. 09-03-121, 98 pp. www.ecy.wa.gov/biblio/0903121.html.



Figure 1. PSAMP 10 long-term temporal sediment monitoring stations in Puget Sound.

| Station | Location             | Target<br>(NAD 83, decimal degrees) |          |  |  |
|---------|----------------------|-------------------------------------|----------|--|--|
| Dunion  | 200000               | Longitude                           | Latitude |  |  |
| 3       | Strait of Georgia    | 122.97842                           | 48.87025 |  |  |
| 4       | Bellingham           | 122.53820                           | 48.68397 |  |  |
| 21      | Everett              | 122.24283                           | 47.98547 |  |  |
| 29      | Shilshole            | 122.45403                           | 47.70075 |  |  |
| 34      | Sinclair Inlet       | 122.66208                           | 47.54708 |  |  |
| 38      | Point Pully          | 122.39363                           | 47.42833 |  |  |
| 40      | Commencement Bay     | 122.43730                           | 47.26130 |  |  |
| 44      | East Anderson Island | 122.67358                           | 47.16133 |  |  |
| 49      | Budd Inlet           | 122.91347                           | 47.07997 |  |  |
| 13R     | North Hood Canal     | 122.62895                           | 47.83758 |  |  |

Table 1. Location (latitude/longitude) for the 2011 PSAMP Sediment Component Long-term/Temporal Monitoring Element.

Table 2. Parameters measured in Puget Sound sediments for the 2011 PSAMP Sediment Component Long-term/Temporal Monitoring Element.

#### **Field Measurements**

Sediment temperature Salinity of overlying water

#### Macroinvertebrate Abundance

Total Abundance Major Taxa Abundance Taxa Richness Pielou's Evenness Swartz's Dominance Index

#### **Related Parameters**

Grain Size Total organic carbon Table 3. Proposed schedule for completing the 2011 PSAMP Sediment Component Long-term/Temporal Monitoring Element field and laboratory work, EIM data entry, and reports.

| Field and laboratory work                     |   |  |  |  |  |
|---|---|--|--|--|--|
| Field work completed                          |   | April 2011   |  |  |  |
| Laboratory analyses co                        | TOC – July 2011<br>Grain size – September 2011<br>Taxonomy – March 2012 |  |  |  |  |
| Environmental Informa                         | ation System (EI  | M) system  |  |  |  |
| Product                                       | Due date  | Lead Staff   |  |  |  |
| EIM data loaded                               | April 2012  | Sandra Weakland  |  |  |  |
| EIM QA  | May 2012  | Maggie Dutch   |  |  |  |
| EIM complete                                  | June 2012   | Sandra Weakland  |  |  |  |
| Final report: 2011 PSA                        | MP Long-Term  | /Temporal Monitoring   |  |  |  |
| Author lead                                   |   | Maggie Dutch/<br>Sandra Weakland                                   |  |  |  |
| Schedule                                      |   |  |  |  |  |
| Summary statistics,<br>text generated and p   | • •   | June 2012  |  |  |  |
| Draft due to supervi                          | isor  |  |  |  |  |
| Draft due to client/p                         | beer reviewer   | N/A (PSAMP long-   |  |  |  |
| Draft due to externa                          | ll reviewer   | term/temporal report<br>published every 5 <sup>th</sup> year; next |  |  |  |
| Final (all reviews de<br>publications coordin |   | report after 2015 sampling)  |  |  |  |
| Final report due on                           | web   |  |  |  |  |



Figure 2. 73 station locations for Ecology's 2011 PSAMP Spatial/Temporal and Urban Waters Initiative Monitoring in the South Puget Sound Sediment Monitoring Region and Budd Inlet.

| Originating<br>Project | Station | PSAMP<br>/UWI<br>Label ID | Location                   | Longitude | Latitude | PSAMP<br>Spatial/<br>Temporal | UWI | Resampled<br>PSAMP/<br>NOAA | Dioxin/<br>Furan | Bar<br>coding |
|------------------------|---------|---------------------------|----------------------------|-----------|----------|-------------------------------|-----|-----------------------------|------------------|---------------|
| PSAMP Spatial          | 4       | PS00004                   | Carr Inlet                 | 122.6712  | 47.3601  | Х                             |     |                             |                  | Х             |
| PSAMP Spatial          | 22      | PS00022                   | Case Inlet                 | 122.8167  | 47.2263  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 30      | PS00030                   | East<br>Anderson<br>Island | 122.6720  | 47.1540  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 36      | PS00036                   | Totten Inlet               | 123.0168  | 47.1365  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 44      | PS00044                   | Nisqually<br>Reach         | 122.7283  | 47.1179  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 46      | PS00046                   | East<br>Anderson<br>Island | 122.6625  | 47.1863  | Х                             |     |                             |                  | Х             |
| PSAMP Spatial          | 52      | PS00052                   | Nisqually<br>Reach         | 122.7805  | 47.1706  | Х                             |     |                             |                  | Х             |
| PSAMP Spatial          | 54      | PS00054                   | Case Inlet                 | 122.8670  | 47.2798  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 76      | PS00076                   | Case Inlet                 | 122.8414  | 47.2257  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 78      | PS00078                   | Drayton<br>Passage         | 122.7497  | 47.1595  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 86      | PS00086                   | Carr Inlet                 | 122.7104  | 47.2494  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 102     | PS00102                   | Pickering<br>Passage       | 122.8554  | 47.3049  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 110     | PS00110                   | Carr Inlet                 | 122.6572  | 47.2390  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 132     | PS00132                   | Hale<br>Passage            | 122.5936  | 47.2699  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 142     | PS00142                   | Carr Inlet                 | 122.6251  | 47.2211  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 146     | PS00146                   | Case Inlet                 | 122.8147  | 47.3920  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 150     | PS00150                   | Carr Inlet                 | 122.7240  | 47.2813  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 164     | PS00164                   | Oakland<br>Bay             | 123.0620  | 47.2250  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 204     | PS00204                   | Pickering<br>Passage       | 122.9024  | 47.2039  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 206     | PS00206                   | East<br>Anderson<br>Island | 122.6223  | 47.1524  | Х                             |     |                             |                  | Х             |
| PSAMP Spatial          | 238     | PS00238                   | Drayton<br>Passage         | 122.7208  | 47.2428  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 326     | PS00326                   | Drayton<br>Passage         | 122.7438  | 47.1657  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 348     | PS00348                   | Hale<br>Passage            | 122.6711  | 47.2760  | Х                             |     |                             |                  | Х             |
| PSAMP Spatial          | 358     | PS00358                   | East<br>Anderson<br>Island | 122.5859  | 47.1903  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 374     | PS00374                   | East<br>Anderson<br>Island | 122.6658  | 47.1461  | Х                             |     |                             |                  |               |
| PSAMP Spatial          | 388     | PS00388                   | Nisqually<br>Reach         | 122.7205  | 47.1297  | Х                             |     |                             |                  |               |

Table 4. Station information for Ecology's 2011 PSAMP Spatial/Temporal and Urban Waters Initiative Monitoring Programs, and other monitoring activities.

| Originating<br>Project | Station | PSAMP<br>/UWI<br>Label ID | Location           | Longitude | Latitude | PSAMP<br>Spatial/<br>Temporal | UWI | Resampled<br>PSAMP/<br>NOAA | Dioxin/<br>Furan | Bar<br>coding |
|------------------------|---------|---------------------------|--------------------|-----------|----------|-------------------------------|-----|-----------------------------|------------------|---------------|
| PSAMP Spatial          | 508     | PS00508                   | Port of<br>Shelton | 123.0786  | 47.2119  | Х                             |     |                             |                  | Х             |
| PSAMP Spatial          | 636     | PS00636                   | Port of<br>Shelton | 123.0836  | 47.2076  | Х                             |     |                             | Х                |               |
| PSAMP Spatial          | 12      | PSUW012                   | Budd Inlet         | 122.9071  | 47.1241  | Х                             | Х   |                             | Х                |               |
| PSAMP Spatial          | 20      | PSUW020                   | Budd Inlet         | 122.9147  | 47.0815  | Х                             | Х   |                             | Х                |               |
| PSAMP Spatial          | 84      | PSUW084                   | Budd Inlet         | 122.9307  | 47.1001  | Х                             | Х   |                             | Х                |               |
| PSAMP Spatial          | 100     | PSUW100                   | Port of<br>Olympia | 122.8978  | 47.0624  | Х                             | Х   |                             |                  |               |
| PSAMP Spatial          | 116     | PSUW116                   | Budd Inlet         | 122.9109  | 47.1313  | Х                             | Х   |                             | Х                |               |
| PSAMP Spatial          | 140     | PSUW140                   | Budd Inlet         | 122.9093  | 47.1224  | Х                             | Х   |                             | Х                |               |
| PSAMP Spatial          | 148     | PSUW148                   | Budd Inlet         | 122.9116  | 47.0988  | Х                             | Х   |                             | Х                |               |
| PSAMP Spatial          | 228     | PSUW228                   | Port of<br>Olympia | 122.9090  | 47.0568  | Х                             | Х   |                             |                  |               |
| PSAMP Spatial          | 244     | PSUW244                   | Budd Inlet         | 122.9206  | 47.1459  | Х                             | Х   |                             | Х                |               |
| PSAMP Spatial          | 268     | PSUW268                   | Budd Inlet         | 122.9031  | 47.1106  | Х                             | Х   |                             | Х                | Х             |
| PSAMP Spatial          | 300     | PSUW300                   | Port of<br>Olympia | 122.9055  | 47.0526  | Х                             | Х   |                             | Х                |               |
| PSAMP Spatial          | 556     | PSUW556                   | Port of<br>Olympia | 122.9036  | 47.0451  | Х                             | Х   |                             | Х                |               |
| PSAMP/NOAA             | 227     | PSNO227                   | Port of<br>Shelton | 123.0841  | 47.2126  | Х                             |     | Х                           | Х                | Х             |
| PSAMP/NOAA             | 228     | PSNO228                   | Port of<br>Shelton | 123.0828  | 47.2097  | Х                             |     | Х                           |                  |               |
| PSAMP/NOAA             | 229     | PSNO229                   | Port of<br>Shelton | 123.0839  | 47.2124  | Х                             |     | Х                           |                  |               |
| PSAMP/NOAA             | 230     | PSNO230                   | Oakland<br>Bay     | 123.0793  | 47.2088  | Х                             |     | Х                           |                  |               |
| PSAMP/NOAA             | 231     | PSNO231                   | Oakland<br>Bay     | 123.0630  | 47.2194  | Х                             |     | Х                           |                  |               |
| PSAMP/NOAA             | 232     | PSNO232                   | Oakland<br>Bay     | 123.0615  | 47.2207  | Х                             |     | Х                           | Х                |               |
| PSAMP/NOAA             | 233     | PSNO233                   | Totten Inlet       | 123.0044  | 47.1553  | Х                             |     | Х                           |                  |               |
| PSAMP/NOAA             | 234     | PSNO234                   | Totten Inlet       | 123.0385  | 47.1160  | Х                             |     | Х                           |                  | Х             |
| PSAMP/NOAA             | 235     | PSNO235                   | Totten Inlet       | 122.9775  | 47.1532  | Х                             |     | Х                           |                  |               |
| PSAMP/NOAA             | 238     | PSNO238                   | Eld Inlet          | 122.9571  | 47.1133  | Х                             |     | Х                           |                  |               |
| PSAMP/NOAA             | 239     | PSNO239                   | Eld Inlet          | 122.9604  | 47.1222  | Х                             |     | Х                           |                  |               |
| PSAMP/NOAA             | 240     | PSNO240                   | Eld Inlet          | 122.9804  | 47.0994  | Х                             |     | Х                           |                  |               |
| PSAMP/NOAA             | 248     | PSNO248                   | Henderson<br>Inlet | 122.8355  | 47.1429  | Х                             |     | Х                           |                  | Х             |
| PSAMP/NOAA             | 249     | PSNO249                   | Henderson<br>Inlet | 122.8358  | 47.1351  | Х                             |     | Х                           |                  |               |
| PSAMP/NOAA             | 250     | PSNO250                   | Henderson<br>Inlet | 122.8413  | 47.1445  | Х                             |     | Х                           |                  |               |
| PSAMP/NOAA             | 266     | PSNO266                   | Hale<br>Passage    | 122.6458  | 47.2692  |                               |     |                             |                  | Х             |
| PSAMP/NOAA             | 236     | UWNO236                   | Budd Inlet         | 122.8970  | 47.1142  |                               | Х   | Х                           | Х                |               |
| PSAMP/NOAA             | 237     | UWNO237                   | Budd Inlet         | 122.9138  | 47.1293  |                               | Х   | Х                           |                  | Х             |
| PSAMP/NOAA             | 241     | UWNO241                   | Budd Inlet         | 122.9145  | 47.1355  |                               | Х   | Х                           | Х                |               |

| Originating<br>Project   | Station  | PSAMP<br>/UWI<br>Label ID | Location           | Longitude | Latitude | PSAMP<br>Spatial/<br>Temporal | UWI | Resampled<br>PSAMP/<br>NOAA | Dioxin/<br>Furan | Bar<br>coding |
|--------------------------|----------|---------------------------|--------------------|-----------|----------|-------------------------------|-----|-----------------------------|------------------|---------------|
| PSAMP/NOAA               | 242      | UWNO242                   | Port of<br>Olympia | 122.8974  | 47.0529  |                               | Х   | Х                           | Х                |               |
| PSAMP/NOAA               | 243      | UWNO243                   | Port of<br>Olympia | 122.8959  | 47.0516  |                               | Х   | Х                           |                  |               |
| PSAMP/NOAA               | 244      | UWNO244                   | Port of<br>Olympia | 122.9091  | 47.0575  |                               | Х   | Х                           |                  |               |
| Urban Waters             | 40056    | UW40056                   | Budd Inlet         | 122.9027  | 47.0646  |                               | Х   |                             | Х                |               |
| Urban Waters             | 40216    | UW40216                   | Budd Inlet         | 122.9161  | 47.0992  |                               | Х   |                             | Х                |               |
| Urban Waters             | 40272    | UW40272                   | Budd Inlet         | 122.9057  | 47.1263  |                               | Х   |                             | Х                |               |
| Urban Waters             | 40528    | UW40528                   | Budd Inlet         | 122.9157  | 47.1193  |                               | Х   |                             | Х                |               |
| Urban Waters             | 40728    | UW40728                   | Budd Inlet         | 122.9088  | 47.0891  |                               | Х   |                             |                  |               |
| Urban Waters             | 40984    | UW40984                   | Budd Inlet         | 122.9099  | 47.0807  |                               | Х   |                             | Х                |               |
| Urban Waters             | 41040    | UW41040                   | Budd Inlet         | 122.8942  | 47.1055  |                               | Х   |                             | Х                |               |
| Urban Waters             | 41240    | UW41240                   | Budd Inlet         | 122.9120  | 47.0964  |                               | Х   |                             | Х                |               |
| Urban Waters             | 41296    | UW41296                   | Budd Inlet         | 122.8960  | 47.0985  |                               | Х   |                             | Х                |               |
| Urban Waters             | 41552    | UW41552                   | Budd Inlet         | 122.9004  | 47.1178  |                               | Х   |                             | Х                |               |
| Urban Waters             | 41680    | UW41680                   | Budd Inlet         | 122.9229  | 47.1351  |                               | Х   |                             | Х                |               |
| Urban Waters             | 41752    | UW41752                   | Budd Inlet         | 122.9250  | 47.1043  |                               | Х   |                             | Х                |               |
| Urban Waters             | BI-42704 | UW42704                   | Budd Inlet         | 122.9199  | 47.1298  |                               |     |                             | Х                |               |
| Urban Waters             | BI-42776 | UW42776                   | Budd Inlet         | 122.9246  | 47.0888  |                               |     |                             | Х                |               |
| Urban Waters             | BI-43088 | UW43088                   | Budd Inlet         | 122.9091  | 47.1127  |                               |     |                             | Х                |               |
| Urban Waters             | BI-43216 | UW43216                   | Budd Inlet         | 122.9198  | 47.1412  |                               |     |                             | Х                |               |
| Toxic Cleanup<br>Program | BI-S7    |                           | Budd Inlet         | 122.9132  | 47.0591  |                               |     |                             | Х                |               |
| Toxic Cleanup<br>Program | BI-S30   |                           | Budd Inlet         | 122.8946  | 47.0477  |                               |     |                             | Х                |               |
| Toxic Cleanup<br>Program | OB-10    |                           | Oakland<br>Bay     | 123.0496  | 47.2376  |                               |     |                             | Х                |               |
| Toxic Cleanup<br>Program | OB-12.5  |                           | Oakland<br>Bay     | 123.0353  | 47.2513  |                               |     |                             | X                |               |
|                          |          |                           |                    |           | count:   | 55                            | 30  | 21                          | 35               | 12            |

Table 5. Parameters measured in Puget Sound sediments for the 2011 PSAMP Sediment Component Spatial/Temporal Monitoring Element and Urban Waters Initiative.

#### Field Measurements

Sediment temperature Salinity of overlying water

#### **Toxicity Parameters**

Amphipod Survival (solid phase) Urchin Fertilization (porewater)

#### *Macroinvertebrate Abundance*

Total Abundance Major Taxa Abundance Taxa Richness Pielou's Evenness Swartz's Dominance Index

#### **Related Parameters**

Grain Size Total organic carbon

#### **Metals**

#### **Priority Pollutant Metals**

Arsenic Cadmium Chromium Copper Lead Mercury Nickel Selenium Silver Zinc

#### **Element** Tin

#### **Organics**

Chlorinated Alkenes Hexachlorobutadiene **Chlorinated and Nitro-Substituted Phenols** Pentachlorophenol

#### Chlorinated Aromatic Chemicals

1,2,4-Trichlorobenzene 1,2-Dichlorobenzene 1,3-Dichlorobenzene 1,4-Dichlorobenzene 2-Chloronaphthalene Hexachlorobenzene

#### **Chlorinated Pesticides**

2.4'-DDD 2,4'-DDE 2,4'-DDT 4,4'-DDD 4.4'-DDE 4,4'-DDT Aldrin Cis-Chlordane (Alpha-Chlordane) Dieldrin Endosulfan I Endosulfan II Endosulfan Sulfate Endrin Endrin Aldehyde Endrin Ketone Gamma-BHC (Lindane) Heptachlor Heptachlor Epoxide Mirex Oxychlordane Toxaphene Trans-Chlordane (Gamma)

#### Polynuclear Aromatic Hydrocarbons

#### *LPAHs* 1,6,7-Trimethylnaphthalene 1-Methylnaphthalene 1-Methylphenanthrene 2,6-Dimethylnaphthalene

2-Methylnaphthalene 2-Methylphenanthrene Acenaphthene Acenaphthylene Anthracene Biphenyl Dibenzothiophene Fluorene Naphthalene Phenanthrene Retene *Calculated values:* total LPAHs

#### **HPAHs**

Benzo(a)anthracene Benzo(a)pyrene Benzo(b)fluoranthene Benzo(e)pyrene Benzo(g,h,i)perylene Benzo(k)fluoranthene Chrysene Dibenzo(a,h)anthracene Fluoranthene Indeno(1,2,3-c,d)pyrene Perylene Pyrene *Calculated values:* total HPAH total Benzofluoranthenes

## Miscellaneous Extractable

Chemicals Benzoic Acid Benzyl Alcohol Beta-coprostanol Carbazole Cholesterol Dibenzofuran Isophorone

#### **Organonitrogen Chemicals** Caffeine N-Nitrosodiphenylamine

| Phenols<br>2,4-Dimethylphenol<br>2-Methylphenol<br>4-Methylphenol              | PBDE-154<br>PBDE- 183<br>PBDE- 184<br>PBDE-191   | PCB-52<br>PCB-66<br>PCB-77<br>PCB-101   |
|--|--|---|
| Phenol<br>Phenol, 4-Nonyl-   | PBDE-209<br>Polychlorinated                      | PCB-105<br>PCB-118<br>PCB-126   |
| <b>Phthalate Esters</b><br>Bis(2-Ethylhexyl) Phthalate<br>Butylbenzylphthalate | Biphenyls  | PCB-128<br>PCB-138<br>PCB-153   |
| Diethylphthalate<br>Dimethylphthalate<br>Di-N-Butylphthalate                   | PCB-1016<br>PCB-1221<br>PCB-1232                 | PCB-169<br>PCB-170<br>PCB-180   |
| Di-N-Octyl Phthalate Polybrominated  | PCB-1242<br>PCB-1248<br>PCB-1254                 | PCB-187<br>PCB-195<br>PCB-206   |
| <b>Diphenylethers</b><br>PBDE- 47<br>PBDE– 49                                  | PCB-1260<br>PCB-1262<br>PCB-1268                 | PCB-209<br>Added in 2000  |
| PBDE- 66<br>PBDE- 71<br>PBDE- 99<br>PBDE-100<br>PBDE- 138<br>PBDE-153          | Congeners<br>PCB-8<br>PCB-18<br>PCB-28<br>PCB-44 | Bisphenol A<br>Tri(2-chloroethyl)phosphate<br>(TCEP)<br>Triclosan<br>Triethyl citrate |

Table 6. Proposed schedule for completing the 2011 PSAMP Sediment Component Spatial/Temporal Monitoring Element and Urban Waters Initiative field and laboratory work, data entry into EIM, and reports.

| Field and laboratory work                        |  |  |  |  |  |
|--|--|--|--|--|--|
| Field work completed                             |  | June 2011  |  |  |  |
| Laboratory analyses co                           | TOC – July 2011<br>Grain size – September 2011<br>Toxicity – March 2012<br>Taxonomy – March 2012<br>Chemistry – March 2012 |  |  |  |  |
| Environmental Information                        | ation System (EIM  | ) system   |  |  |  |
| Product  | Due date   | Lead Staff                                       |  |  |  |
| EIM data loaded                                  | April 2012   | Sandra Weakland                                  |  |  |  |
| EIM QA   | May 2012   | Maggie Dutch                                     |  |  |  |
| EIM complete                                     | June 2012  | Sandra Weakland                                  |  |  |  |
| Final report: 2011 PSA<br>Initiative: Budd Inlet | AMP Spatial/Temp   | oral and Urban Waters                            |  |  |  |
| Author lead                                      |  | Maggie Dutch (PSAMP)/<br>Valerie Partridge (UWI) |  |  |  |
| Schedule   |  |  |  |  |  |
|  | ics, graphics, and nd posted to web  | June 2012  |  |  |  |
| Draft due to sup                                 | ervisor  | September 2014                                   |  |  |  |
| Draft due to clie                                | nt/peer reviewer   | October 2014                                     |  |  |  |
| Draft due to exte                                | ernal reviewer   | November 2014                                    |  |  |  |
| Final (all review<br>publications coo            | ,  | December 2014                                    |  |  |  |
| 1  |  |  |  |  |  |

Table 7. 121 nitrogen and organophosphorous pesticides, pyrethroids, and herbicides measured in Puget Sound sediments for the 2011 PSAMP Sediment Component Spatial/Temporal Monitoring Element and Urban Waters Initiative.

Nitrogen Containing Pesticides Acetochlor Alachlor Atrazine Benfluralin (Benefin) Bromacil **Butachlor Butylate** Carboxin Chlorothalonil (Daconil) Chlorpropham Cyanazine Cycloate Di-allate (Avadex) Diazoxon Dichlobenil Diphenamid Eptam Ethalfluralin (Sonalan) Fenarimol Fenvalerate Fipronil **Fipronil Disulfinyl** Fipronil Sulfide Fipronil Sulfone Fluridone Hexazinone Metalaxyl Methyl Paraoxon Metolachlor Metribuzin Napropamide Norflurazon Oryzalin Oxyfluorfen Pebulate Pendimethalin Prometon Prometryn Pronamide (Kerb) Propachlor (Ramrod) Propargite Propazine Simazine Simetryn Tebuthiuron

Thiobencarb (Benthiocarb) Triadimefon Triallate Tricyclazole Trifluralin **Organophosphorous** Pesticides Azinphos Ethyl Azinphos-methyl Chlorpyrifos Chlorpyrifos O.A. Coumaphos Diazinon Dichlorvos (DDVP) Dimethoate Disulfoton (Di-Syston) Disulfoton sulfone EPN Ethion Ethoprop Fenamiphos Fenamiphos Sulfone Fonofos Imidan Malathion Methidathion Methyl Chlorpyrifos Methyl Parathion Mevinphos Monocrotophos Naled Oxydisulfoton Parathion Phorate Phorate O.A. Phosmet O.A. Sulfotepp Terbacil Tetrachlorvinphos (Gardona) Tokuthion Trichloronate

#### **Pyrethroids**

beta-Cypermethrin Bifenthrin Butoxide, Piperonyl cis-Permethrin Deltamethrin lambda-Cyhalothrin MGK264 Phenothrin Resmethrin Tralomethrin trans-Permethrin

#### Herbicides

2,4,6-Trichlorophenol 3.5-Dichlorobenzoic Acid 4-Nitrophenol Clopyralid 2,4,5-Trichlorophenol Dicamba 2,3,4,6-Tetrachlorophenol **MCPP MCPA** Dichlorprop Bromoxynil 2,4-D 2,3,4,5-Tetrachlorophenol Triclopyr Pentachlorophenol Silvex 2,4,5-T 2.4-DB Dinoseb Bentazon Ioxynil Picloram Dacthal Acifluorfen, sodium salt **Diclofop-Methyl** Chloramben

Table 8. Sample volumes and preservation for laboratory analysis for nitrogen containing and organophosphorus pesticides, pyrethroids, and herbicides.

| Parameter   | Size of<br>Sample | Container   | Preservation                              | Maximum<br>Holding Time   |
|---|-------------------|---|---|---|
| Nitrogen containing and<br>organophosphorus<br>pesticides, pyrethroids, and<br>herbicides | 8 oz              | 1 8-oz certified organic-<br>free wide-mouth glass jar<br>with Teflon-lined lid | Store and transport all samples at 0-6°C. | 14 days from collection to<br>extraction at 0-6°C, 1 year if<br>frozen at ≤18°C; 40 days from<br>extraction to analysis |

Table 9. Laboratory analysis and reporting requirements for nitrogen containing and organophosphorus pesticides, pyrethroids, and herbicides.

| Parameter  | Expected<br>Range of<br>Results | Extraction<br>Method | Clean-Up<br>Method | Analysis<br>Method   | Technique/ Instrument  | Required<br>Reporting Limit                                   |
|--|---------------------------------|----------------------|--------------------|--|--|---|
| Nitrogen<br>containing and<br>organophosphorus<br>pesticides,<br>pyrethroids | Unknown                         |                      |                    | SW-846 Method 8270   | Gas Chromatography Mass<br>Spectrometer – GC/MS  | 50-1000 ug/kg<br>(nitrogen),<br>10-1000 ug/kg<br>(organophos) |
| Herbicides   | Unknown                         |                      |                    | SW-846 Method 8270<br>and 8151 with<br>modifications as per<br>EPA Region 10<br>Herbicide Procedure for<br>Soils and Drinking and<br>Raw Source Waters | Gas Chromatography Mass<br>Spectrometer – GC/MS or Gas<br>Chromatography Electron<br>Capture Detector – GC/ECD | 10-1000 ug/kg   |

Table 10. Field and laboratory measurement quality objectives for sediment nitrogen containing and organophosphorus pesticides, pyrethroids, and herbicides.

| Parameter   | Field Blank         | Field<br>Replicate<br>(Split Sample)       | Analytical (Laboratory) Replicate  | Laboratory<br>Control<br>Sample | Reference<br>Material <sup>1</sup> | Method Blank   | Matrix<br>Spike (and<br>Matrix<br>Spike<br>Duplicates) | Surrogate Spike   |
|---|---------------------|--|--|---------------------------------|------------------------------------|--|--|---|
| Measurement<br>Frequency  |                     | Duplicate<br>analysis for 5%<br>of samples | Triplicate analysis/batch of 20 samples<br>for grain size and TOC. Duplicate<br>analysis/batch for metals and organics<br>samples. | 1/batch of 20                   | 1/batch of 20                      | 1/batch of 20  | 1/batch of 20  | every organics sample,<br>blank, and QC sample<br>(minimum of 3 for<br>neutrals, 3 for acids) |
| MQO measured  |                     | RPD  | RSD or RPD   | % recovery<br>limits            | % recovery<br>limits               | comparison of analyte concentration<br>in blank to quantification limit                                  | % recovery<br>limits                                   | % recovery limits   |
| Nitrogen containing and<br>organophosphorus<br>pesticides, pyrethroids,<br>and herbicides | RPD <u>&lt;</u> 20% | RPD <u>&lt;</u> 20%                        | Compound specific RPD ≤ 40%  | 50-150                          | NA                                 | Analyte concentration $<$ MDL; if $\ge$ MDL, lowest analyte concn. must be $\ge 10x$ method blank concn. | 50-150   | 50-150  |

Method Blanks - analyzed to assess possible laboratory contamination of samples associated with all stages of preparation and analysis of sample extracts.

Surrogate Spike Compounds - a type of check standard that is added to each sample in a known amount prior to extraction or purging.

Analytical replicates - provide precision information on the actual samples; useful in assessing potential samples heterogeneity and matrix effects.

Matrix Spikes - percent recoveries of matrix spikes are reported, should include a wide range of representative analyte types, compounds should be spiked about 5x the concentration of compounds in the sample or 5x the quantification limit.

Laboratory Control Samples - sometimes called check standards or laboratory control samples, are method blanks spiked with surrogate compounds and analytes; useful in verifying acceptable method performance prior to and during routine analysis of samples.

**Reference Materials -** a material or substance whose property values are sufficiently well established to be used for calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

**Certified Reference Material -** a reference material, provided by standard setting organizations such as NIST, CRM, etc., accompanied by or traceable to a certificate or other documentation that is issues by a certifying body.

Table 11. Possible parameters measured in Puget Sound sediments for concentrations of pharmaceuticals and personal care products (PPCPs), and perfluorinated chemicals (PFCs), in Puget Sound sediments.

*The final parameter list will be determined by the lead investigators at the University of Washington, Tacoma, at a later date.* 

#### **Field Measurements**

Sediment temperature Salinity of overlying water

#### **PPCPs**

List 1 - Acid Extraction in **Positive Ionization** Acetaminophen Ampicillin 1 Azithromycin Caffeine Carbadox Carbamazepine Cefotaxime Ciprofloxacin Clarithromycin Clinafloxacin Cloxacillin Dehydronifedipine Digoxigenin Digoxin Diltiazem 1,7-Dimethylxanthine Diphenhydramine Enrofloxacin Erythromycin-H20 Flumequine Fluoxetine Lincomycin Lomefloxacin Miconazole Norfloxacin Norgestimate Ofloxacin Ormetoprim Oxacillin

Oxolinic acid Penicillin G Penicillin V Roxithromycin Sarafloxacin Sulfachloropyridazine Sulfadiazine Sulfadimethoxine Sulfamerazine Sulfamethazine Sulfamethizole Sulfamethoxazole Sulfanilamide Sulfathiazole Thiabendazole Trimethoprim Tylosin Virginiamycin

# List 2 - Tetracyclines in Positive Ionization

Anhydrochlortetracycline Anhydrotetracycline Chlortetracycline Demeclocycline Doxycycline 4-Epianhydrochlortetracycline 4-Epichlortetracycline 4-Epichlortetracycline 4-Epitetracycline Isochlortetracycline Minocycline Oxytetracycline Tetracycline

#### List 3 - Acid Extraction in Negative Ionization Bisphenol A Furosemide Gemfibrozil Glipizide Glyburide Hydrochlorothiazide 2-hydroxy-ibuprofen Ibuprofen Naproxen Triclocarban Triclosan Warfarin

List 4 - Basic Extraction in **Positive Ionization** Albuterol Amphetamine Atenolol Atorvastatin Cimetidine Clonidine Codeine Cotinine Enalapril Hydrocodone Metformin Oxycodone Ranitidine Triamterene

#### List 5 - Acid Extraction in Positive Ionization Alprazolam Amitriptyline Amlodipine Benzoylecgonine

Benztropine Betamethasone Cocaine DEET Desmethyldiltiazem Diazepam Fluocinonide Fluticasone propionate Hydrocortisone 10-hydroxy-amitriptyline Meprobamate Methylprednisolone Metoprolol Norfluoxetine Norverapamil Paroxetine Prednisolone Prednisone

Promethazine Propoxyphene Propranolol Sertraline Simvastatin Theophylline Trenbolone Trenbolone acetate Valsartan Verapamil

#### **Perfluorinated Chemicals**

#### **Carboxylic Acids**

Perfluorobutanoate (PFBA) Perfluoropentanoate (PFPeA) Perfluorohexanoate (PFHxA) Perfluoroheptanoate (PFHpA) Perfluorooctanoate (PFOA) Perfluorononanoate (PFNA) Perfluorodecanoate (PFDA) Perfluoroundecanoate (PFUnA) Perfluorododecanoate (PFDoA) Perfluorotridecanoic Acid (PFTrDA) Perfluorotetradecanoic Acid (PFTeDA) Perfluorohexadecanoic Acid (PFHxDA) Perfluorooctadecanoic Acid (PFODA)

#### **Sulphonic Acids**

Perfluorobutanesulfonate (PFBS) Perfluorohexanesulfonate (PFHxS) Perfluorooctanesulfonate (PFOS) Perfluorodecanesulfonate (PFDS) Table 12. Sample volumes and preservation for laboratory analysis for pharmaceuticals and personal care products (PPCPs) and perfluorinated chemicals (PFCs).

| Parameter  | Size of<br>Sample | Container   | Preservation  | Maximum<br>Holding Time  |
|--|-------------------|---|---|--|
| Pharmaceuticals<br>and Personal Care<br>Products (PPCPs) | 8 oz              | 8 oz HDPE<br>internally<br>certified by<br>contract lab | Wrap in aluminum foil and place in ice<br>chest with dry ice immediately after field<br>collection. Freeze as soon as possible.<br>Store in dark at less than -10°C until<br>analyzed | * Freezing encouraged to minimize degradation.<br>Extract within 48 hours if not frozen or within 7<br>days of collection if frozen. Extract within 48<br>hours of removal from freezer. Analyze extracts<br>within 40 days of extraction. |
| Perfluorinated<br>Chemicals (PFCs)                       | 8 oz              | 8 oz HDPE<br>internally<br>certified by<br>contract lab | Refrigerate at 4°C±2°C (CAS)  | * 14 days to extraction (CAS)  |

\* These are suggested holding times only. Formal holding time studies have not been performed or published for this analysis.

Table 13. Laboratory analysis and reporting requirements for pharmaceuticals and personal care products (PPCPs) and perfluorinated chemicals (PFCs).

| Parameter  | Expected<br>Range of<br>Results | Extraction<br>Method  | Clean-Up<br>Method   | Analysis<br>Method                     | Technique/ Instrument   | Required<br>Reporting<br>Limit |
|--|---------------------------------|---|--|--|---|--------------------------------|
| Pharmaceuticals<br>and Personal Care<br>Products (PPCPs) | Unknown                         | Sonication with aqueous buffered<br>acetonitrile and pure acetonitrile,<br>concentrate then dilute with ultra<br>pure water.  | Solid-phase<br>extraction<br>cartridge then<br>filtered      | USEPA<br>1694                          | HPLC/ESI-MS/MS. High performance<br>liquid chromatography with triple<br>quadrupole mass spectrometer in<br>positive and negative electrospray<br>ionization modes using isotope dilution<br>and internal standard quantitation<br>techniques | 1-1,000<br>μg/kg dry<br>weight |
| Perfluorinated<br>Chemicals (PFCs)                       | Unknown                         | Shake extraction with dilute acetic<br>acid solution then methanolic<br>ammonium hydroxide solution.<br>Combine supernatants and treat<br>with ultra pure carbon powder and<br>diluted with ultra pure water. | Weak anion<br>exchange<br>sorbent solid-<br>phase extraction | MLA-041.<br>Internal<br>Axys<br>method | HPLC/ESI-MS/MS. High performance<br>liquid chromatography with triple<br>quadrupole mass spectrometer in<br>negative electrospray ionization mode<br>using internal standard.   | 0.1 μg/kg<br>dry weight        |

Table 14. Field and laboratory measurement quality objectives for pharmaceuticals and personal care products (PPCPs) and perfluorinated chemicals (PFCs).

| Parameter  | Field Blank         | Field<br>Replicate<br>(Split Sample)       | Analytical (Laboratory) Replicate  | Laboratory<br>Control<br>Sample | Reference<br>Material <sup>1</sup> | Method Blank   | Matrix<br>Spike (and<br>Matrix<br>Spike<br>Duplicates) | Surrogate Spike   |
|--|---------------------|--|--|---------------------------------|------------------------------------|--|--|---|
| Measurement<br>Frequency                                 |                     | Duplicate<br>analysis for 5%<br>of samples | Triplicate analysis/batch of 20 samples<br>for grain size and TOC. Duplicate<br>analysis/batch for metals and organics<br>samples. | 1/batch of 20                   | 1/batch of 20                      | 1/batch of 20  | 1/batch of 20  | every organics sample,<br>blank, and QC sample<br>(minimum of 3 for<br>neutrals, 3 for acids) |
| MQO measured   | RPD                 | RPD  | RSD or RPD   | % recovery<br>limits            | % recovery<br>limits               | comparison of analyte concentration<br>in blank to quantification limit  | % recovery<br>limits                                   | % recovery limits   |
| Pharmaceuticals<br>and Personal Care<br>Products (PPCPs) | RPD ≤ 20%           | RPD ≤ 20%                                  | Compound specific RPD $\leq 40\%$  | compound<br>specific            | NA                                 | Analyte concentration $<$ MDL; if $\ge$<br>MDL, lowest analyte concn. must be<br>$\ge 10x$ method blank concn. | NA   | compound specific   |
| Perfluorinated<br>Chemicals (PFCs)                       | RPD <u>&lt;</u> 20% | RPD ≤ 20%                                  | Compound specific RPD ≤ 40%  | compound<br>specific            | NA                                 | Analyte concentration $<$ MDL; if $\ge$<br>MDL, lowest analyte concn. must be<br>$\ge 10x$ method blank concn. | Recovery<br>compound<br>specific;<br>RPDs<40           | compound specific   |

Method Blanks - analyzed to assess possible laboratory contamination of samples associated with all stages of preparation and analysis of sample extracts.

Surrogate Spike Compounds - a type of check standard that is added to each sample in a known amount prior to extraction or purging.

Analytical replicates - provide precision information on the actual samples; useful in assessing potential samples heterogeneity and matrix effects.

Matrix Spikes - percent recoveries of matrix spikes are reported, should include a wide range of representative analyte types, compounds should be spiked about 5x the concentration of compounds in the sample or 5x the quantification limit.

Laboratory Control Samples - sometimes called check standards or laboratory control samples, are method blanks spiked with surrogate compounds and analytes; useful in verifying acceptable method performance prior to and during routine analysis of samples.

**Reference Materials -** a material or substance whose property values are sufficiently well established to be used for calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

**Batch:** a collection of 20 or fewer samples undergoing the same analyses at the same time.

**MDL:** Method Detection Limit

**RPD:** Relative Percent Difference

**RSD:** Relative Standard Deviation

NA: Not Applicable



Figure 3. Station locations for Ecology's 2011 sediment sampling in Budd Inlet and Oakland Bay for dioxins and furans.

Table 15. Parameters measured in Budd Inlet and Oakland Bay sediments to determine dioxin and furan concentrations.

#### Field Measurements

Sediment temperature Salinity of overlying water

#### **Related Parameters**

Grain size Total organic carbon

#### **Organics**

#### **Dioxin and Furan congeners**

PCDD 2,3,7,8-TCDD 1 2 3 7 8 PeCD

1,2,3,7,8-PeCDD 1,2,3,4,7,8-HxCDD 1,2,3,6,7,8-HxCDD 1,2,3,7,8,9-HxCDD 1,2,3,4,6,7,8-HpCDD 1,2,3,4,6,7,8,9-OCDD

PCDF

2,3,7,8-TCDF 1,2,3,7,8-PeCDF 2,3,4,7,8-PeCDF 1,2,3,4,7,8-HxCDF 1,2,3,6,7,8-HxCDF 1,2,3,7,8,9-HxCDF 2,3,4,6,7,8-HxCDF 1,2,3,4,6,7,8-HpCDF 1,2,3,4,6,7,8,9-HpCDF 1,2,3,4,6,7,8,9-OCDF

| Parameter             | Size of<br>Sample | Container  | Preservation    | Maximum<br>Holding Time                         |
|-----------------------|-------------------|--|-----------------|---|
| Dioxins<br>and Furans | 8 oz              | 8 oz certified organic-free wide-mouth glass jar with Teflon-lined lid | Freeze at -10°C | 1 year pre-extraction<br>1 year post-extraction |

Table 16. Sample volumes and preservation for laboratory analysis for dioxin and furan samples.

Table 17. Laboratory analysis and reporting requirements for dioxin and furan samples.

| Parameter                        | Expected<br>Range of<br>Results | Extraction<br>Method   | Clean-Up<br>Method                            | Analysis<br>Method  | Technique/<br>Instrument | Required<br>Reporting<br>Limit |
|----------------------------------|---------------------------------|------------------------|---|---|--------------------------|--------------------------------|
| Dioxins<br>and Furans<br>(ng/kg) | < 0.5 -<br>< 500                | As specified by method | All necessary<br>(silica, alumina,<br>carbon) | SW846 Method 1613B<br>(EPA, 1994;<br>especially Sections 11-14) | HRGC /<br>HRMS           | Varies –<br>See Table 18       |

| PCDD/F congener      | Sediment<br>Target EQL<br>(ng/Kg<br>dry weight) |
|----------------------|---|
| PCDD                 |   |
| 2,3,7,8-TCDD         | 1.0   |
| 1,2,3,7,8-PeCDD      | 1.0   |
| 1,2,3,4,7,8-HxCDD    | 2.5   |
| 1,2,3,6,7,8-HxCDD    | 2.5   |
| 1,2,3,7,8,9-HxCDD    | 2.5   |
| 1,2,3,4,6,7,8-HpCDD  | 2.5   |
| 1,2,3,4,6,7,8,9-OCDD | 5.0   |
| PCDF                 |   |
| 2,3,7,8-TCDF         | 1.0   |
| 1,2,3,7,8-PeCDF      | 2.5   |
| 2,3,4,7,8-PeCDF      | 1.0   |
| 1,2,3,4,7,8-HxCDF    | 2.5   |
| 1,2,3,6,7,8-HxCDF    | 2.5   |
| 1,2,3,7,8,9-HxCDF    | 2.5   |
| 2,3,4,6,7,8-HxCDF    | 2.5   |
| 1,2,3,4,6,7,8-HpCDF  | 2.5   |
| 1,2,3,4,7,8,9-HpCDF  | 2.5   |
| 1,2,3,4,6,7,8,9-OCDF | 5.0   |

Table 18. Target estimated quantitation limits (EQLs) for sediment samples collected from Budd Inlet and Oakland Bay.
| Parameter  | Initial<br>Calibration<br>(r) | Continuing<br>calibration<br>(% recovery) | EQL               | Field | l blanks |     | oratory<br>ks/batch | &/c<br>spił | duplicates<br>or matrix<br>ces/batch<br>6 RPD) |     | LCS or SRM<br>(% recovery)  |
|--|-------------------------------|---|-------------------|-------|----------|-----|---------------------|-------------|--|-----|---|
|  |                               |   | MQO               | No.   | MQO      | No. | MQO                 | No.         | MQO  | No. | $MQO^3$   |
| Dioxins/furans<br>Individual<br>congeners<br>(ng/kg<br>dry weight) | See Method<br>(EPA, 1994)     | See Method<br>(EPA, 1994)                 | Varies<br>1.0-5.0 |       |          | 1   | <0.5RL              | 1           | < 50   | 1   | Specified by<br>method or within<br>2 standard<br>deviations of<br>actual |

Table 19. Measurement quality objectives for field and laboratory quality control samples (per batch  $\leq$  20 samples).

Table 20. Proposed schedule for Ecology's 2011 study of PCDD/Fs in surface sediments of Budd Inlet and Oakland Bay.

| Field and laboratory w             | ork                  |   |
|------------------------------------|----------------------|---|
| Field work completed               |                      | June 2011   |
|                                    |                      | TOC – July 2011   |
| Laboratory analyses co             | ompleted             | Grain size – September 2011<br>Chemistry – September 2011 |
| Environmental Informa              | ation System (EIM    |   |
| Product                            | Due date             | Lead Staff  |
| EIM data loaded                    | March 2012           | Tom Gries   |
| EIM QA                             | April 2012           | David Osterberg   |
| EIM complete                       | May 2012             | Tom Gries   |
| Final report: 2011 Urb             | oan Waters Initiativ | e: Bellingham Bay   |
| Author lead                        |                      | Tom Gries   |
| Schedule                           |                      |   |
| Draft due to sup                   | ervisor              | November 2011   |
| Draft due to clie                  | nt/peer reviewer     | December 2011   |
| Draft due to exte                  | ernal reviewer       | January 2012  |
| Final (all review publications coo |                      | March 2012  |
| Final report due                   | on web               | April 2012  |



Figure 4. Station locations for Ecology's 2011 collection of DNA barcode samples in Budd Inlet and Oakland Bay.

Table 21. PSAMP Spatial/Temporal, PSAMP Long Term/Temporal, Focus Studies, and Urban Waters Initiative sediment sampling schedule (1997-2024).

|   | _    |      |      |      |      |               |               |        |         |        |      | _    |      |                 |      | _    |      |      |      |      | _      |       |        |                 |      |      |      |      |
|---|------|------|------|------|------|---------------|---------------|--------|---------|--------|------|------|------|-----------------|------|------|------|------|------|------|--------|-------|--------|-----------------|------|------|------|------|
|   |      |      |      |      |      | Num           | ber of S      | Sample | es Coll | lected |      |      |      |                 |      |      |      |      |      | Num  | ber of | Sampl | es Exp | ected           |      |      |      |      |
| year sampled:   | 1997 | 1998 | 1999 | 2000 | 2001 | 2002          | 2003          | 2004   | 2005    | 2006   | 2007 | 2008 | 2009 | 2010            | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017   | 2018  | 2019   | 2020            | 2021 | 2022 | 2023 | 2024 |
| Spatial/Temporal Monitoring                             |      |      |      |      |      |               |               |        |         |        |      |      |      |                 |      |      |      |      |      |      |        |       |        |                 |      |      |      |      |
| San Juan Archipelago                                    |      |      |      |      |      | 0.0 (0)       | 1             |        |         |        |      |      |      |                 |      | 40   |      |      |      |      |        |       |        |                 |      | 40   |      |      |
| Eastern Strait of Juan de Fuca                          |      |      |      |      |      | 90 (8<br>+9 ( | 1 new<br>old) |        |         |        |      |      |      |                 |      |      | 40   |      |      |      |        |       |        |                 |      |      | 40   |      |
| Admiralty Inlet   |      |      |      |      |      |               | 010)          |        |         |        |      |      |      |                 |      |      |      | 40   |      |      |        |       |        |                 |      |      |      | 40   |
| Strait of Georgia and Bellingham                        | 100  |      |      |      |      |               |               |        |         | 40     |      |      |      |                 |      |      |      |      |      | 40   |        |       |        |                 |      |      |      |      |
| Whidbey Basin   |      |      |      |      |      |               |               |        |         |        | 40   |      |      |                 |      |      |      |      |      |      | 40     |       |        |                 |      |      |      |      |
| Central Sound (north)                                   |      | 100  |      |      |      |               |               |        |         |        |      | 30   |      |                 |      |      |      |      |      |      |        | 40    |        |                 |      |      |      |      |
| Central Sound (south)                                   |      | 100  |      |      |      |               |               |        |         |        |      |      | 50   |                 |      |      |      |      |      |      |        |       | 40     |                 |      |      |      |      |
| South Sound   |      |      | 100  |      |      |               |               |        |         |        |      |      |      |                 | 43   |      |      |      |      |      |        |       |        |                 | 40   |      |      |      |
| Hood Canal  |      |      | 100  |      |      |               |               | 30     |         |        |      |      |      |                 |      |      |      |      | 40   |      |        |       |        |                 |      |      |      |      |
| Urban Waters Initiative                                 |      |      |      |      |      |               |               |        |         |        |      |      |      |                 |      |      |      |      |      |      |        |       |        |                 |      |      |      |      |
| Elliott Bay/Lower Duwamish                              |      |      |      |      |      |               |               |        |         |        | 30   |      |      |                 |      |      | 30   |      |      |      |        |       | 30     |                 |      |      |      |      |
| Commencement Bay  |      |      |      |      |      |               |               |        |         |        |      | 30   |      |                 |      |      |      | 30   |      |      |        |       |        | 30              |      |      |      |      |
| Bainbridge Basin,<br>including Sinclair and Dyes Inlets |      |      |      |      |      |               |               |        |         |        |      |      | 30   |                 |      |      |      |      | 30   |      |        |       |        |                 | 30   |      |      |      |
| Bellingham Bay  |      |      |      |      |      |               |               |        |         |        |      |      |      | 30              |      |      |      |      |      | 30   |        |       |        |                 |      | 30   |      |      |
| Budd Inlet  |      |      |      |      |      |               |               |        |         |        |      |      |      |                 | 30   |      |      |      |      |      | 30     |       |        |                 |      |      | 30   |      |
| Everett Harbor/Port Gardner                             |      |      |      |      |      |               |               |        |         |        |      |      |      |                 |      | 30   |      |      |      |      |        | 30    |        |                 |      |      |      | 30   |
| Long Term/Temporal Monitoring                           | 30   | 30   | 30   | 30+  | 30   | 30            | 30            | 30     | 30+     | 30     | 30   | 30   | 30   | 30+             | 30   | 30   | 30   | 30   | 30+  | 30   | 30     | 30    | 30     | 30+             | 30   | 30   | 30   | 30   |
| Focus Study/Special Projects                            |      |      |      |      |      |               |               |        |         |        |      |      |      | 40 <sup>1</sup> |      |      |      |      |      |      |        |       |        | 30 <sup>2</sup> |      |      |      |      |

\* 30: Grain Size/Total Organic Carbon/Benthos collected; 30+ = Grain Size/Total Organic Carbon/Benthos/Chemistry collected Focus Studies:

40<sup>1</sup>: 2010 - Pharmaceuticals and Personal Care Products (PPCPs), Perfluorinated Chemicals (PCs) at 10 Long-term/Temporal stations and at 30 UWI Bellingham Bay stations. 30<sup>2</sup>: 2020 - Focus study to be determined.

# Appendix A. Field Navigation Report, including final sampling coordinates, for April 2011 PSAMP Long-Term/Temporal monitoring stations in Puget Sound.

|                |      |       |             |      |           | Station    | Target      | DGPS       | Location          | Dist.  |      | Meter | Predicted  | Predicted | Predicted | GPS     | [ ] ]      |
|----------------|------|-------|-------------|------|-----------|------------|-------------|------------|-------------------|--------|------|-------|------------|-----------|-----------|---------|------------|
|                |      |       |             |      |           |            | 1983        |            | D (2-m. accuracy) | to     |      | Wheel | Tide (m.): | Mudline   | Mudline   | Status  | 1          |
| Station        | Sta. | Sta.  | C=chemistry | Grab | Date      |            | Minutes     |            | ecimal Minutes    | Target | GPS  | Depth | Nearest    | Depth, m. | Depth, m. | HDOP    | Comments   |
| No.            | Rep. | No.   | B=benthos   | No.  | Dato      | Latitude   | Longitude   | Latitude   | Longitude         | m.     | Time | m.    | Station    | (MLLW)    | 2010      | <2 good | Connormo   |
| Station 49     | 1    | 49-1  | C, B        | 1    | 7-Apr-11  | 47 04.7980 | 122 54.8080 | 47 04.7982 | 122 54.8079       | 0.4    | 0803 | 9.3   | 4.0        | -5.3      | -5.0      | 1.1     | unwt. grab |
| Inner Budd     | 4    | 49-1  | C           | 2    |           | 47 04.7980 | 122 54.8080 | 47 04.7985 | 122 54.8079       | 0.9    | 0816 | 9.1   | 4.0        | -5.1      | -5.4      | 1.4     | same hole  |
| Inlet          | 4    | 49-1  | С, В        | 3    |           | 47 04.7980 | 122 54.8080 | 47 04.7972 | 122 54.8078       | 1.5    | 0828 | 9.2   | 4.0        | -5.2      | -5.2      | 1.0     |            |
|                | 2    | 49-2  | С, В        | 4    |           | 47 04.8017 | 122 54.7987 | 47 04.8020 | 122 54.7992       | 0.8    | 0849 | 9.0   | 3.9        | -5.1      | -5.1      | 1.0     |            |
|                | 3    | 49-3  | С, В        | 5    |           | 47 04.8053 | 122 54.8080 | 47 04.8051 | 122 54.8076       | 0.6    | 0907 | 9.0   | 3.8        | -5.2      | -5.3      | 1.0     |            |
| Station 44     | 1    | 44-1  | С, В        | 1    | 7-Apr-11  | 47 09.6800 | 122 40.4150 | 47 09.6800 | 122 40.4160       | 1.3    | 1119 | 22.5  | 1.9        | -20.6     | -21.0     | 0.9     |            |
| East Ander-    | 4    | 44-1  | С, В        | 2    |           | 47 09.6800 | 122 40.4150 | 47 09.6798 | 122 40.4153       | 0.5    | 1128 | 22.2  | 1.7        | -20.5     | -21.0     | 0.9     | weighted   |
| son Island     | 2    | 44-3  | С, В        | 3    |           | 47 09.7411 | 122 40.4150 | 47 09.7408 | 122 40.4155       | 0.8    | 1145 | 25.7  | 1.5        | -24.2     | -16.8     | 1.2     | grab       |
|                | 3    | 44-2  | С, В        | 4    |           | 47 09.6494 | 122 40.3890 | 47 09.6495 | 122 40.3883       | 0.9    | 1157 | 25.8  | 1.3        | -24.5     | -31.6     | 1.9     |            |
| Station 40     | 1    | 40-1  | C, B        | 1    | 7-Apr-11  | 47 15.6780 | 122 26.2380 | 47 15.6775 | 122 26.2376       | 1.1    | 1411 | 10.3  | -0.2       | -10.5     | -10.1     | 1.1     |            |
| Entrance to    | 4    | 40-1  | С, В        | 2    |           | 47 15.6780 | 122 26.2380 | 47 15.6783 | 122 26.2379       | 0.6    | 1425 | 10.1  | -0.2       | -10.3     | -10.0     | 0.8     | weighted   |
| Thea Foss      | 2    | 40-2  | С, В        | 3    |           | 47 15.6847 | 122 26.2380 | 47 15.6846 | 122 26.2378       | 0.3    | 1448 | 10.5  | -0.1       | -10.6     | -10.0     | 0.9     | grab       |
| Waterway       | 3    | 40-3  | С, В        | 4    |           | 47 15.6814 | 122 26.2294 | 47 15.6807 | 122 26.2283       | 1.9    | 1507 | 10.8  | 0.1        | -10.8     | -10.4     | 0.9     | 1 reject   |
| Station 38     | 1    | 38-1  | С, В        | 1    | 8-Apr-11  | 47 25.7000 | 122 23.6180 | 47 25.6994 | 122 23.6175       | 1.3    | 0811 | 203   | 3.1        | -200      | -200      | 1.4     |            |
| Pt. Pully      | 4    | 38-1  | С, В        | 2    |           | 47 25.7000 | 122 23.6180 | 47 25.6998 | 122 23.6174       | 0.8    | 0828 | 203   | 3.1        | -200      | -200      | 1.0     | weighted   |
| (3-Tree Pt.)   | 2    | 38-2  | С, В        | 3    |           | 47 25.7134 | 122 23.5838 | 47 25.7126 | 122 23.5843       | 1.6    | 0847 | 203   | 3.0        | -200      | -200      | 1.0     | grab       |
|                | 3    | 38-3  | С, В        | 4    |           | 47 25.6733 | 122 23.6180 | 47 25.6732 | 122 23.6176       | 0.5    | 0906 | 203   | 2.9        | -200      | -200      | 1.0     |            |
| Station 34     | 1    | 34-1  | С, В        | 1    | 8-Apr-11  | 47 32.8250 | 122 39.7250 | 47 32.8248 | 122 39.7252       | 0.4    | 1137 | 11.2  | 1.6        | -9.6      | -9.4      | 1.2     |            |
| Sinclair Inlet | 4    | 34-1  | С, В        | 2    |           | 47 32.8250 | 122 39.7250 | 47 32.8250 | 122 39.7245       | 0.6    | 1149 | 11.2  | 1.5        | -9.8      | -9.2      | 2.1     | unwt. grab |
|                | 2    | 34-2  | С, В        | 3    |           | 47 32.8212 | 122 39.7152 | 47 32.8208 | 122 39.7155       | 0.8    | 1236 | 10.2  | 0.9        | -9.3      | -9.4      | 1.4     | 1 reject   |
|                | 3    | 34-3  | С, В        | 4    |           | 47 32.8288 | 122 39.7348 | 47 32.8284 | 122 39.7357       | 1.4    | 1252 | 10.0  | 0.7        | -9.3      | -9.4      | 1.1     | 1          |
| Station 29     | 1    | 29-1  | С, В        | 1    | 8-Apr-11  | 47 42.0450 | 122 27.2420 | 47 42.0453 | 122 27.2420       | 0.6    | 1525 | 201   | -0.1       | -201      | -201      | 1.3     |            |
| Shilshole      | 4    | 29-1  | С, В        | 2    |           | 47 42.0450 | 122 27.2420 | 47 42.0452 | 122 27.2428       | 1.1    | 1543 | 201   | 0.0        | -201      | -201      | 0.9     | weighted   |
|                | 2    | 29-2  | С, В        | 3    |           | 47 42.0322 | 122 27.2091 | 47 42.0315 | 122 27.2111       | 2.8    | 1600 | 201   | 0.2        | -201      | -200      | 0.8     | grab       |
|                | 3    | 29-3  | С, В        | 4    |           | 47 42.0706 | 122 27.2420 | 47 42.0709 | 122 27.2422       | 0.6    | 1619 | 201   | 0.3        | -201      | -200      | 1.0     |            |
| Station 4      | 1    | 04-1  | С, В        | 1    | 15-Apr-11 | 48 41.0380 | 122 32.2920 | 48 41.0376 | 122 32.2916       | 0.9    | 0857 | 25.0  | 0.7        | -24.3     | -24.1     | 1.0     | unwt. grab |
| Bellingham     | 4    | 04-1  | С, В        | 2    |           | 48 41.0380 | 122 32.2920 | 48 41.0380 | 122 32.2915       | 0.6    | 0910 | 25.2  | 0.7        | -24.5     | -24.1     | 1.0     |            |
|                | 2    | 04-2  | С, В        | 3    |           | 48 41.0425 | 122 32.2803 | 48 41.0424 | 122 32.2799       | 0.5    | 0936 | 25.3  | 0.7        | -24.6     | -23.9     | 0.8     |            |
|                | 3    | 04-3  | С, В        | 4    |           | 48 41.0291 | 122 32.2920 | 48 41.0292 | 122 32.2910       | 1.2    | 1003 | 25.0  | 0.7        | -24.3     | -24.1     | 0.9     |            |
| Station 3      | 1    | 03-1  | С, В        | 1    | 15-Apr-11 | 48 52.2150 | 122 58.7050 | 48 52.2220 | 122 58.7031       | 13.2   | 1309 | 224   | 1.4        | -223      | -222      | 1.5     |            |
| Straits of     | 4    | 03-1  | C, B        | 2    |           | 48 52.2150 | 122 58.7050 | 48 52.2167 | 122 58.7033       | 3.8    | 1326 | 224   | 1.5        | -222      | -222      | 1.1     | weighted   |
| Georgia        | 2    | 03-2  | C, B        | 3    |           | 48 52.1974 | 122 58.6585 | 48 52.1989 | 122 58.6579       | 2.9    | 1403 | 225   | 1.8        | -223      | -222      | 1.0     | grab       |
|                | 3    | 03-3  | C, B        | 4    |           | 48 52.1797 | 122 58.7050 | 48 52.1805 | 122 58.7037       | 2.2    | 1427 | 226   | 1.9        | -224      | -222      | 0.9     | 1 reject   |
| Sta. 13R       | 1    | 13R-1 | C, B        | 1    | 18-Apr-11 | 47 50.2550 | 122 37.7370 | 47 50.2548 | 122 37.7380       | 1.3    | 1050 | 20.3  | -0.4       | -20.7     | -20.7     | 0.9     |            |
| North Hood     | 4    | 13R-1 | C, B        | 2    |           | 47 50.2550 | 122 37.7370 | 47 50.2557 | 122 37.7361       | 1.7    | 1104 | 20.3  | -0.5       | -20.8     | -21.0     | 1.3     | weighted   |
| Canal          | 2    | 13R-2 | C, B        | 3    |           | 47 50.2507 | 122 37.7482 | 47 50.2496 | 122 37.7488       | 2.2    | 1146 | 20.8  | -0.6       | -21.4     | -22.4     | 1.4     | grab       |
|                | 2    | 13R-2 | C           | 4    |           | 47 50.2507 | 122 37.7482 | 47 50.2499 | 122 37.7475       | 1.7    | 1202 | 21.6  | -0.6       | -22.2     | 21.6      | 1.3     | 1 reject   |
| 01-11-11-01    | 3    | 13R-3 | C, B        | 5    | 40.4      | 47 50.2507 | 122 37.7258 | 47 50.2519 | 122 37.7250       | 2.4    | 1214 | 19.8  | -0.5       | -20.3     | -21.6     | 1.1     |            |
| Station 21     | 1    | 21-1  | C, B        | 1    | 18-Apr-11 | 47 59.1280 | 122 14.5700 | 47 59.1281 | 122 14.5697       | 0.4    | 1538 | 23.0  | 2.1        | -20.9     | -20.9     | 1.0     |            |
| Everett        | 4    | 21-1  | C, B        | 2    |           | 47 59.1280 | 122 14.5700 | 47 59.1287 | 122 14.5708       | 1.6    | 1554 | 22.9  | 2.3        | -20.6     | -21.1     | 0.9     | weighted   |
|                | 2    | 21-2  | C, B        | 4    |           | 47 59.1323 | 122 14.5588 | 47 59.1321 | 122 14.5580       | 1.1    | 1611 | 19.6  | 2.5        | -17.1     | -20.9     | 1.0     | grab       |
|                | 3    | 21-3  | С, В        | Э    |           | 47 59.1323 | 122 14.5812 | 47 59.1335 | 122 14.5803       | 2.5    | 1629 | 22.8  | 2.8        | -20.0     | -25.5     | 0.9     |            |

## Appendix B. Field Navigation Report, including final sampling coordinates, for June 2011 PSAMP Spatial/Temporal, Urban Waters Initiative monitoring stations, and associated special projects in Puget Sound.

| Station<br>No.                 | Rep | Date     | NAI        | n Target<br>D 1983<br>I Minutes<br>Longitude | DGPS L<br>Trimble 1<br>(1-m. ac<br>NAD 1983, De<br>Latitude | NT300D<br>curacy) | GPS<br>Time | Dist.<br>to<br>Target<br>m. | Meter<br>Wheel<br>Depth<br>m. | Predicted<br>Tide (m.):<br>Nearest<br>Station | Predicted<br>Mudline<br>Depth, m.<br>(MLLW) | GPS<br>Status<br>HDOP<br><2<br>good | Comments          |
|--------------------------------|-----|----------|------------|--|---|-------------------|-------------|-----------------------------|-------------------------------|---|---|-------------------------------------|-------------------|
| UW40528,<br>BI-40528           | 1   | 1-Jun-11 | 47 07.1568 | 122 54.9438                                  | 47 07.1570  | 122 54.9438       | 0930        | 0.4                         | 15.1                          | 1.04  | -14.1                                       | 1.4                                 |                   |
|                                | 2   |          |            |  | 47 07.1564  | 122 54.9437       | 0946        | 0.8                         | 15.2                          | 0.79  | -14.4                                       | 1.4                                 |                   |
|                                | 3   |          |            |  | 47 07.1564  | 122 54.9435       | 1002        | 0.8                         | 15.0                          | 0.53  | -14.5                                       | 1.4                                 |                   |
|                                | 4   |          |            |  | 47 07.1564  | 122 54.9433       | 1015        | 1.0                         | 14.5                          | 0.33  | -14.2                                       | 1.4                                 |                   |
| UW41552,<br>BI-41552           | 1   | 1-Jun-11 | 47 07.0650 | 122 54.0258                                  | 47 07.0645  | 122 54.0251       | 1111        | 1.3                         | 9.6                           | -0.34   | -9.9  | 1.0                                 |                   |
|                                | 2   |          |            |  | 47 07.0651  | 122 54.0254       | 1124        | 0.5                         | 9.6                           | -0.44   | -10.0                                       | 1.0                                 |                   |
|                                | 3   |          |            |  | 47 07.0656  | 122 54.0260       | 1141        | 1.1                         | 9.4                           | -0.54   | -9.9  | 1.3                                 |                   |
|                                | 4   |          |            |  | 47 07.0651  | 122 54.0249       | 1155        | 1.2                         | 9.5                           | -0.59   | -10.1                                       | 1.3                                 |                   |
| UWNO236,<br>BI-236             | 1   | 1-Jun-11 | 47 06.8542 | 122 53.8171                                  | 47 06.8537  | 122 53.8170       | 1314        | 0.9                         | 7.0                           | -0.32   | -7.3  | 1.2                                 |                   |
|                                | 2   |          |            |  | 47 06.8536  | 122 53.8171       | 1327        | 1.1                         | 7.0                           | -0.20   | -7.2  | 1.2                                 |                   |
|                                | 3   |          |            |  | 47 06.8544  | 122 53.8169       | 1341        | 0.4                         | 7.2                           | -0.03   | -7.2  | 1.2                                 |                   |
|                                | 4   |          |            |  | 47 06.8541  | 122 53.8155       | 1355        | 2.0                         | 7.5                           | 0.16  | -7.3  | 1.2                                 |                   |
| PSUW300,<br>BI-300,<br>FS00004 | 1   | 1-Jun-11 | 47 03.1574 | 122 54.3453                                  | 47 03.1574  | 122 54.3453       | 1455        | 0.0                         | 13.8                          | 1.07  | -12.7                                       | 0.8                                 |                   |
|                                | 2   |          |            |  | 47 03.1584  | 122 54.3453       | 1508        | 1.9                         | 14.0                          | 1.32  | -12.7                                       | 0.9                                 | target under dock |
|                                | 3   |          |            |  | 47 03.1560  | 122 54.3434       | 1519        | 3.5                         | 13.9                          | 1.53  | -12.4                                       | 0.8                                 | moved 18 m west   |
|                                | 4   |          |            |  | 47 03.1554  | 122 54.3434       | 1530        | 4.4                         | 13.4                          | 1.72  | -11.7                                       | 0.9                                 |                   |
|                                | 5   |          |            |  | 47 03.1565  | 122 54.3444       | 1542        | 2.0                         | 14.1                          | 1.93  | -12.2                                       | 0.8                                 |                   |
| UWNO24,<br>BI-242              | 1   | 1-Jun-11 | 47 03.1717 | 122 53.8417                                  | 47 03.1718  | 122 53.8401       | 1636        | 2.0                         | 6.0                           | 2.87  | -3.1  | 0.9                                 |                   |
|                                | 2   |          |            |  | 47 03.1724  | 122 53.8413       | 1649        | 1.4                         | 6.2                           | 3.07  | -3.1  | 0.9                                 |                   |
|                                | 3   |          |            |  | 47 03.1719  | 122 53.8411       | 1702        | 0.8                         | 6.6                           | 3.27  | -3.3  | 1.0                                 |                   |

| Station<br>No.       | Rep | Date     | NAI<br>Decima | <u>n Target</u><br>D 1983<br>Il Minutes | <u>DGPS L</u><br>Trimble l<br>(1-m. ac<br>NAD 1983, De | NT300D<br>ccuracy)<br>ccimal Minutes | GPS<br>Time | Dist.<br>to<br>Target<br>m. | Meter<br>Wheel<br>Depth<br>m. | Predicted<br>Tide (m.):<br>Nearest<br>Station | Predicted<br>Mudline<br>Depth, m.<br>(MLLW) | GPS<br>Status<br>HDOP<br><2<br>good | Comments               |
|----------------------|-----|----------|---------------|---|--|--------------------------------------|-------------|-----------------------------|-------------------------------|---|---|-------------------------------------|------------------------|
|                      |     |          | Latitude      | Longitude                               | Latitude   | Longitude                            |             |                             |                               |   |   | Ũ                                   |                        |
|                      | 4   |          |               |   | 47 03.1721   | 122 53.8415                          | 1715        | 0.8                         | 6.7                           | 3.45  | -3.3  | 1.0                                 |                        |
| BI-S 30              | 1   | 1-Jun-11 | 47 02.8620    | 122 53.6760                             | 47 02.8621   | 122 53.6775                          | 1736        | 1.9                         | 2.5                           | 3.75  | 1.3   | 1.1                                 |                        |
| UW40216,<br>BI-40216 | 1   | 2-Jun-11 | 47 05.9502    | 122 54.9666                             | 47 05.9504   | 122 54.9659                          | 0953        | 1.0                         | 12.2                          | 1.18  | -11.0                                       | 1.4                                 |                        |
|                      | 2   |          |               |   | 47 05.9507   | 122 54.9664                          | 1007        | 1.0                         | 11.9                          | 0.95  | -11.0                                       | 1.4                                 |                        |
|                      | 3   |          |               |   | 47 05.9516   | 122 54.9676                          | 1019        | 2.9                         | 11.9                          | 0.75  | -11.2                                       | 1.5                                 |                        |
|                      | 4   |          |               |   | 47 05.9500   | 122 54.9665                          | 1030        | 0.4                         | 11.8                          | 0.56  | -11.2                                       | 1.3                                 |                        |
| PSUW084,<br>BI-84    | 1   | 2-Jun-11 | 47 06.0048    | 122 55.8390                             | 47 06.0045   | 122 55.8386                          | 1118        | 0.8                         | 8.0                           | -0.13   | -8.1  | 1.0                                 |                        |
|                      | 2   |          |               |   | 47 06.0044   | 122 55.8395                          | 1129        | 1.0                         | 8.0                           | -0.26   | -8.3  | 1.3                                 |                        |
|                      | 3   |          |               |   | 47 06.0056   | 122 55.8381                          | 1144        | 1.9                         | 7.7                           | -0.42   | -8.1  | 1.3                                 |                        |
|                      | 4   |          |               |   | 47 06.0051   | 122 55.8406                          | 1158        | 2.1                         | 7.2                           | -0.53   | -7.7  | 1.2                                 |                        |
| BI-42776             | 1   | 2-Jun-11 | 47 05.3280    | 122 55.4760                             | 47 05.3275   | 122 55.4750                          | 1332        | 1.6                         | 9.6                           | -0.59   | -10.2                                       | 1.3                                 |                        |
| PSUW020,<br>BI-20    | 1   | 2-Jun-11 | 47 04.8924    | 122 54.8838                             | 47 04.8923   | 122 54.8822                          | 1349        | 2.0                         | 5.4                           | -0.44   | -5.8  | 1.6                                 |                        |
|                      | 2   |          |               |   | 47 04.8925   | 122 54.8849                          | 1401        | 1.4                         | 5.5                           | -0.32   | -5.8  | 1.3                                 |                        |
|                      | 3   |          |               |   | 47 04.8929   | 122 54.8831                          | 1415        | 1.3                         | 5.6                           | -0.16   | -5.8  | 1.2                                 |                        |
|                      | 4   |          |               |   | 47 04.8920   | 122 54.8832                          | 1427        | 1.1                         | 5.8                           | 0.02  | -5.8  | 1.2                                 |                        |
| PSUW228,<br>FS-00001 | 1   | 2-Jun-11 | 47 03.4080    | 122 54.5394                             | 47 03.4078   | 122 54.5396                          | 1522        | 0.4                         | 7.8                           | 0.90  | -6.9  | 0.9                                 |                        |
|                      | 2   |          |               |   | 47 03.4079   | 122 54.5398                          | 1538        | 0.5                         | 8.0                           | 1.16  | -6.8  | 0.9                                 |                        |
|                      | 3   |          |               |   | 47 03.4085   | 122 54.5388                          | 1549        | 1.2                         | 8.8                           | 1.38  | -7.4  | 0.9                                 |                        |
|                      | 4   |          |               |   | 47 03.4078   | 122 54.5384                          | 1603        | 1.3                         | 9.1                           | 1.64  | -7.5  | 0.9                                 |                        |
| UWNO244              | 1   | 2-Jun-11 | 47 03.4500    | 122 54.5500                             | 47 03.4504   | 122 54.5500                          | 1636        | 0.7                         | 4.0                           | 2.28  | -1.7  | 0.9                                 |                        |
|                      | 2   |          |               |   | 47 03.4500   | 122 54.5497                          | 1646        | 0.4                         | 4.2                           | 2.45  | -1.8  | 0.9                                 |                        |
|                      | 3   |          |               |   | 47 03.4496   | 122 54.5507                          | 1657        | 1.2                         | 4.3                           | 2.66  | -1.6  | 1.0                                 |                        |
| BI-S7                | 1   | 2-Jun-11 | 47 03.5445    | 122 54.7488                             | 47 03.5445   | 122 54.7488                          | 1726        | 0.0                         | 3.1                           | 3.15  | 0.0   | 1.1                                 | rocks, moved 55 m east |
| UWNO243<br>FS00003   | 1   | 2-Jun-11 | 47 03.0983    | 122 53.7533                             | 47 03.0979   | 122 53.7522                          | 1807        | 1.6                         | 4.9                           | 3.75  | -1.2  | 1.0                                 |                        |
|                      | 2   |          |               |   | 47 03.0980   | 122 53.7516                          | 1824        | 2.2                         | 4.9                           | 3.93  | -1.0  | 1.2                                 |                        |

| Station<br>No.       | Rep | Date     | NAI        | n <u>Target</u><br>D 1983<br>I Minutes | <u>DGPS L</u><br>Trimble l<br>(1-m. ac<br>NAD 1983, De | NT300D<br>curacy) | GPS<br>Time | Dist.<br>to<br>Target<br>m. | Meter<br>Wheel<br>Depth<br>m. | Predicted<br>Tide (m.):<br>Nearest<br>Station | Predicted<br>Mudline<br>Depth, m.<br>(MLLW) | GPS<br>Status<br>HDOP<br><2 | Comments            |
|----------------------|-----|----------|------------|--|--|-------------------|-------------|-----------------------------|-------------------------------|---|---|-----------------------------|---------------------|
|                      |     |          | Latitude   | Longitude                              | Latitude   | Longitude         |             |                             |                               |   | · · · ·                                     | good                        |                     |
|                      | 3   |          |            |  | 47 03.0981   | 122 53.7528       | 1835        | 0.7                         | 5.3                           | 4.04  | -1.3  | 1.0                         |                     |
|                      | 4   |          |            |  | 47 03.0988   | 122 53.7532       | 1848        | 0.9                         | 5.3                           | 4.15  | -1.2  | 0.9                         |                     |
| PSUW140,<br>BI-140   | 1   | 3-Jun-11 | 47 07.3452 | 122 54.5598                            | 47 07.3450   | 122 54.5603       | 0954        | 0.7                         | 13.7                          | 1.79  | -11.9                                       | 1.4                         |                     |
|                      | 2   |          |            |  | 47 07.3452   | 122 54.5601       | 1005        | 0.4                         | 13.5                          | 1.60  | -11.9                                       | 1.4                         |                     |
|                      | 3   |          |            |  | 47 07.3456   | 122 54.5599       | 1014        | 0.8                         | 13.4                          | 1.45  | -12.0                                       | 1.4                         |                     |
|                      | 4   |          |            |  | 47 07.3461   | 122 54.5587       | 1023        | 2.2                         | 13.2                          | 1.29  | -11.9                                       | 1.3                         |                     |
| PSUW012,<br>BI-12    | 1   | 3-Jun-11 | 47 07.4442 | 122 54.4230                            | 47 07.4448   | 122 54.4235       | 1043        | 1.3                         | 14.1                          | 0.96  | -13.1                                       | 1.0                         |                     |
|                      | 2   |          |            |  | 47 07.4441   | 122 54.4237       | 1051        | 0.9                         | 14.1                          | 0.82  | -13.3                                       | 1.0                         |                     |
|                      | 3   |          |            |  | 47 07.4446   | 122 54.4228       | 1059        | 0.8                         | 14.1                          | 0.71  | -13.4                                       | 1.0                         |                     |
|                      | 4   |          |            |  | 47 07.4437   | 122 54.4226       | 1109        | 1.1                         | 14.1                          | 0.51  | -13.6                                       | 1.0                         |                     |
| PSUW268,<br>BI-268   | 1   | 3-Jun-11 | 47 06.6360 | 122 54.1848                            | 47 06.6355   | 122 54.1850       | 1132        | 1.0                         | 9.9                           | 0.17  | -9.7  | 1.3                         |                     |
|                      | 2   |          |            |  | 47 06.6364   | 122 54.1849       | 1143        | 0.8                         | 9.8                           | 0.01  | -9.8  | 1.3                         |                     |
|                      | 3   |          |            |  | 47 06.6352   | 122 54.1870       | 1157        | 3.2                         | 9.8                           | -0.17   | -10.0                                       | 1.0                         |                     |
|                      | 4   |          |            |  | 47 06.6356   | 122 54.1854       | 1206        | 1.1                         | 9.7                           | -0.27   | -10.0                                       | 0.9                         |                     |
| BI-43088             | 1   | 3-Jun-11 | 47 06.7620 | 122 54.5460                            | 47 06.7611   | 122 54.5460       | 1327        | 1.7                         | 10.9                          | -0.80   | -11.7                                       | 1.2                         |                     |
| UW41040,<br>BI-41040 | 1   | 3-Jun-11 | 47 06.3316 | 122 53.7314                            | 47 06.3316   | 122 53.7314       | 1344        | 0.0                         | 2.9                           | -0.78   | -3.7  | 1.6                         | target high         |
|                      |     |          |            |  | 47 06.3305   | 122 53.7309       | 1402        | 2.1                         | 3.0                           | -0.70   | -3.7  | 1.2                         | rocky intertidal    |
|                      |     |          |            |  | 47 06.3300   | 122 53.7307       | 1413        | 3.1                         | 3.1                           | -0.63   | -3.7  | 1.2                         | moved 100 m<br>west |
|                      |     |          |            |  | 47 06.3308   | 122 53.7302       | 1423        | 2.1                         | 3.1                           | -0.55   | -3.7  | 1.2                         |                     |
| UW40728              | 1   | 3-Jun-11 | 47 05.3436 | 122 54.5262                            | 47 05.3429   | 122 54.5259       | 1440        | 1.4                         | 6.1                           | -0.37   | -6.5  | 0.8                         |                     |
|                      | 2   |          |            |  | 47 05.3439   | 122 54.5269       | 1502        | 1.0                         | 5.9                           | -0.08   | -6.0  | 0.8                         |                     |
|                      | 3   |          |            |  | 47 05.3433   | 122 54.5261       | 1506        | 0.6                         | 6.0                           | 0.04  | -6.0  | 0.8                         |                     |
| UW41296,<br>BI-41296 | 1   | 3-Jun-11 | 47 05.9112 | 122 53.7767                            | 47 05.9112   | 122 53.7767       | 1551        | 0.0                         | 4.7                           | 0.75  | -4.0  | 0.9                         | mooring buoy        |
|                      | 2   |          |            |  | 47 05.9107   | 122 53.7762       | 1602        | 1.1                         | 4.7                           | 0.91  | -3.8  | 0.9                         | on target sta.      |
|                      | 3   |          |            |  | 47 05.9108   | 122 53.7784       | 1610        | 2.3                         | 4.9                           | 1.08  | -3.8  | 1.1                         | moved 18 m west     |

| Station<br>No.       | Rep | Date     | NAI<br>Decima | <u>n Target</u><br>D 1983<br>I Minutes | DGPS L<br>Trimble l<br>(1-m. ac<br>NAD 1983, De | NT300D<br>curacy)<br>cimal Minutes | GPS<br>Time | Dist.<br>to<br>Target<br>m. | Meter<br>Wheel<br>Depth<br>m. | Predicted<br>Tide (m.):<br>Nearest<br>Station | Predicted<br>Mudline<br>Depth, m.<br>(MLLW) | GPS<br>Status<br>HDOP<br><2 | Comments      |
|----------------------|-----|----------|---------------|--|---|------------------------------------|-------------|-----------------------------|-------------------------------|---|---|-----------------------------|---------------|
|                      |     |          | Latitude      | Longitude                              | Latitude  | Longitude                          |             |                             |                               |   |   | good                        |               |
|                      | 4   |          |               |  | 47 05.9112                                      | 122 53.7767                        | 1621        | 0.0                         | 5.0                           | 1.30  | -3.7  | 1.1                         |               |
| UW40056,<br>BI-40056 | 1   | 6-Jun-11 | 47 03.8748    | 122 54.1620                            | 47 03.8748                                      | 122 54.1619                        | 0910        | 0.1                         | 6.4                           | 3.52  | -2.9  | 1.4                         |               |
|                      | 2   |          |               |  | 47 03.8754                                      | 122 54.1615                        | 0920        | 1.3                         | 6.6                           | 3.48  | -3.1  | 1.4                         |               |
|                      | 3   |          |               |  | 47 03.8741                                      | 122 54.1621                        | 0932        | 1.3                         | 6.5                           | 3.44  | -3.1  | 1.4                         |               |
|                      | 4   |          |               |  | 47 03.8753                                      | 122 54.1630                        | 0942        | 1.6                         | 6.4                           | 3.38  | -3.0  | 1.4                         |               |
| UW40984,<br>BI-40984 | 1   | 6-Jun-11 | 47 04.8402    | 122 54.5928                            | 47 04.8409                                      | 122 54.5915                        | 1014        | 2.1                         | 8.0                           | 3.15  | -4.9  | 1.2                         |               |
|                      | 2   |          |               |  | 47 04.8400                                      | 122 54.5930                        | 1024        | 0.4                         | 7.9                           | 3.06  | -4.8  | 1.3                         |               |
|                      | 3   |          |               |  | 47 04.8405                                      | 122 54.5929                        | 1033        | 0.6                         | 8.0                           | 2.97  | -5.0  | 1.0                         |               |
|                      | 4   |          |               |  | 47 04.8405                                      | 122 54.5913                        | 1042        | 2.0                         | 8.0                           | 2.87  | -5.1  | 1.0                         |               |
| UW41240,<br>BI-41240 | 1   | 6-Jun-11 | 47 05.7840    | 122 54.7182                            | 47 05.7839                                      | 122 54.7181                        | 1104        | 0.2                         | 12.8                          | 2.62  | -10.2                                       | 1.0                         |               |
|                      | 2   |          |               |  | 47 05.7842                                      | 122 54.7173                        | 1117        | 1.2                         | 12.5                          | 2.49  | -10.0                                       | 1.3                         |               |
|                      | 3   |          |               |  | 47 05.7841                                      | 122 54.7180                        | 1125        | 0.3                         | 12.6                          | 2.38  | -10.2                                       | 1.3                         |               |
|                      | 4   |          |               |  | 47 05.7840                                      | 122 54.7189                        | 1134        | 0.9                         | 12.4                          | 2.25  | -10.2                                       | 1.3                         |               |
| UW41752,<br>BI-41752 | 1   | 6-Jun-11 | 47 06.2568    | 122 55.4976                            | 47 06.2571                                      | 122 55.4975                        | 1254        | 0.6                         | 7.2                           | 1.12  | -6.1  | 1.2                         |               |
|                      | 2   |          |               |  | 47 06.2570                                      | 122 55.4974                        | 1303        | 0.4                         | 7.1                           | 0.99  | -6.1  | 1.3                         |               |
|                      | 3   |          |               |  | 47 06.2572                                      | 122 55.4974                        | 1312        | 0.8                         | 7.0                           | 0.85  | -6.2  | 1.3                         |               |
|                      | 4   |          |               |  | 47 06.2561                                      | 122 55.4971                        | 1322        | 1.4                         | 7.0                           | 0.71  | -6.3  | 1.3                         |               |
| UW40272,<br>BI-40272 | 1   | 6-Jun-11 | 47 07.5798    | 122 54.3426                            | 47 07.5802                                      | 122 54.3422                        | 1357        | 0.9                         | 19.4                          | 0.26  | -19.1                                       | 1.2                         |               |
|                      | 2   |          |               |  | 47 07.5802                                      | 122 54.3425                        | 1408        | 0.8                         | 19.4                          | 0.15  | -19.3                                       | 1.2                         |               |
|                      | 3   |          |               |  | 47 07.5792                                      | 122 54.3428                        | 1417        | 1.1                         | 19.0                          | 0.04  | -19.0                                       | 1.1                         |               |
|                      | 4   |          |               |  | 47 07.5794                                      | 122 54.3432                        | 1425        | 1.1                         | 19.0                          | -0.03   | -19.0                                       | 1.1                         |               |
| BI-42704             | 1   | 6-Jun-11 | 47 07.7880    | 122 55.1940                            | 47 07.7879                                      | 122 55.1943                        | 1456        | 0.4                         | 24.8                          | -0.27   | -25.1                                       | 1.0                         |               |
| UW41680,<br>BI-41680 | 1   | 6-Jun-11 | 47 08.1203    | 122 55.2951                            | 47 08.1203                                      | 122 55.2951                        | 1537        | 0.0                         | 27.3                          | -0.42   | -27.7                                       | 0.9                         |               |
|                      | 2   |          |               |  | 47 08.1196                                      | 122 55.2945                        | 1547        | 1.5                         | 27.3                          | -0.42   | -27.7                                       | 0.9                         | rocks on sta. |
|                      | 3   |          |               |  | 47 08.1209                                      | 122 55.2962                        | 1554        | 1.8                         | 27.5                          | -0.40   | -27.9                                       | 0.9                         | moved target  |

| Station<br>No.                 | Rep | Date     | NAI<br>Decima | <u>n Target</u><br>D 1983<br>1l Minutes | <u>DGPS L</u><br>Trimble l<br>(1-m. ac<br>NAD 1983, De | NT300D<br>curacy)<br>cimal Minutes | GPS<br>Time | Dist.<br>to<br>Target<br>m. | Meter<br>Wheel<br>Depth<br>m. | Predicted<br>Tide (m.):<br>Nearest<br>Station | Predicted<br>Mudline<br>Depth, m.<br>(MLLW) | GPS<br>Status<br>HDOP<br><2 | Comments     |
|--------------------------------|-----|----------|---------------|---|--|------------------------------------|-------------|-----------------------------|-------------------------------|---|---|-----------------------------|--------------|
|                                |     |          | Latitude      | Longitude                               | Latitude   | Longitude                          |             |                             |                               |   |   | good                        |              |
|                                | 4   |          |               |   | 47 08.1195   | 122 55.2947                        | 1605        | 1.6                         | 27.5                          | -0.37   | -27.9                                       | 1.1                         | 100 m. WNW   |
| BI-43216                       | 1   | 6-Jun-11 | 47 08.4720    | 122 55.1880                             | 47 08.4721   | 122 55.1888                        | 1628        | 1.0                         | 27.2                          | -0.24   | -27.4                                       | 0.9                         |              |
| PSUW556,<br>BI-556,<br>FS00005 | 1   | 7-Jun-11 | 47 02.7076    | 122 54.2875                             | 47 02.7076   | 122 54.2875                        | 0842        | 0.0                         | 6.2                           | 3.17  | -3.0  | 1.1                         |              |
|                                | 2   |          |               |   | 47 02.7073   | 122 54.2864                        | 0859        | 1.5                         | 6.4                           | 3.25  | -3.2  | 1.1                         | moved target |
|                                | 3   |          |               |   | 47 02.7070   | 122 54.2873                        | 0912        | 1.1                         | 6.5                           | 3.30  | -3.2  | 1.4                         | 93 m west    |
|                                | 4   |          |               |   | 47 02.7081   | 122 54.2866                        | 0926        | 1.5                         | 6.6                           | 3.33  | -3.3  | 1.4                         | onto water   |
| PSUW100                        | 1   | 7-Jun-11 | 47 03.7446    | 122 53.8668                             | 47 03.7453   | 122 53.8675                        | 1001        | 1.6                         | 4.9                           | 3.33  | -1.6  | 1.3                         |              |
|                                | 2   |          |               |   | 47 03.7449   | 122 53.8675                        | 1011        | 1.0                         | 5.0                           | 3.31  | -1.7  | 1.3                         |              |
|                                | 3   |          |               |   | 47 03.7445   | 122 53.8670                        | 1026        | 0.3                         | 5.0                           | 3.26  | -1.7  | 1.0                         |              |
| PSUW148,<br>BI-148             | 1   | 7-Jun-11 | 47 05.9250    | 122 54.6966                             | 47 05.9247   | 122 54.6971                        | 1145        | 0.8                         | 12.9                          | 2.71  | -10.2                                       | 0.9                         |              |
|                                | 2   |          |               |   | 47 05.9255   | 122 54.6962                        | 1206        | 1.1                         | 12.6                          | 2.49  | -10.1                                       | 1.3                         |              |
|                                | 3   |          |               |   | 47 05.9251   | 122 54.6958                        | 1223        | 1.0                         | 12.2                          | 2.30  | -9.9  | 1.3                         |              |
| UWNO237                        | 1   | 7-Jun-11 | 47 07.7563    | 122 54.8269                             | 47 07.7561   | 122 54.8274                        | 1357        | 0.7                         | 13.0                          | 1.13  | -11.9                                       | 1.2                         |              |
|                                | 2   |          |               |   | 47 07.7562   | 122 54.8274                        | 1414        | 0.7                         | 12.8                          | 0.93  | -11.9                                       | 1.1                         |              |
|                                | 3   |          |               |   | 47 07.7561   | 122 54.8265                        | 1431        | 0.6                         | 12.2                          | 0.72  | -11.5                                       | 1.0                         |              |
| UWNO241,<br>BI-241             | 1   | 7-Jun-11 | 47 08.1278    | 122 54.8698                             | 47 08.1279   | 122 54.8706                        | 1554        | 1.0                         | 11.6                          | 0.03  | -11.6                                       | 1.1                         |              |
|                                | 2   |          |               |   | 47 08.1276   | 122 54.8691                        | 1610        | 1.0                         | 11.3                          | -0.03   | -11.3                                       | 1.4                         |              |
|                                | 3   |          |               |   | 47 08.1280   | 122 54.8700                        | 1622        | 0.4                         | 11.4                          | -0.05   | -11.5                                       | 0.9                         |              |
|                                | 4   |          |               |   | 47 08.1281   | 122 54.8689                        | 1633        | 1.3                         | 11.4                          | -0.05   | -11.5                                       | 1.0                         |              |
| PS00142                        | 1   | 8-Jun-11 | 47 13.2648    | 122 37.5078                             | 47 13.2662   | 122 37.5084                        | 0930        | 2.7                         | 145                           | 2.66  | -142  | 1.4                         |              |
|                                | 2   |          |               |   | 47 13.2648   | 122 37.5077                        | 0945        | 0.1                         | 145                           | 2.75  | -142  | 1.4                         |              |
|                                | 3   |          |               |   | 47 13.2654   | 122 37.5061                        | 0959        | 2.4                         | 145                           | 2.81  | -142  | 1.3                         |              |
| PS00110                        | 1   | 8-Jun-11 | 47 14.3400    | 122 39.4326                             | 47 14.3401   | 122 39.4333                        | 1031        | 0.9                         | 118                           | 2.86  | -115  | 1.0                         |              |
|                                | 2   |          |               |   | 47 14.3401   | 122 39.4328                        | 1049        | 0.3                         | 118                           | 2.87  | -115  | 1.0                         |              |
|                                | 3   |          |               |   | 47 14.3394   | 122 39.4322                        | 1104        | 1.2                         | 118                           | 2.87  | -115  | 1.0                         |              |
| PS00358                        | 1   | 8-Jun-11 | 47 11.4162    | 122 35.1534                             | 47 11.4166   | 122 35.1534                        | 1219        | 0.7                         | 5.5                           | 2.65  | -2.9  | 1.3                         |              |
|                                | 2   |          |               |   | 47 11.4159   | 122 35.1526                        | 1229        | 1.2                         | 5.1                           | 2.58  | -2.5  | 1.3                         |              |

| Station<br>No. | Rep | Date      | NAI        | n Target<br>D 1983<br>al Minutes<br>Longitude | DGPS L<br>Trimble 1<br>(1-m. ac<br>NAD 1983, De<br>Latitude | NT300D<br>curacy) | GPS<br>Time | Dist.<br>to<br>Target<br>m. | Meter<br>Wheel<br>Depth<br>m. | Predicted<br>Tide (m.):<br>Nearest<br>Station | Predicted<br>Mudline<br>Depth, m.<br>(MLLW) | GPS<br>Status<br>HDOP<br><2<br>good | Comments        |
|----------------|-----|-----------|------------|---|---|-------------------|-------------|-----------------------------|-------------------------------|---|---|-------------------------------------|-----------------|
|                | 3   |           | Luniuu     | Zongitude                                     | 47 11.4161  | 122 35.1538       | 1243        | 0.5                         | 5.4                           | 2.51  | -2.9  | 1.2                                 |                 |
| PS00266        | 1   | 8-Jun-11  | 47 16.1512 | 122 38.7442                                   | 47 16.1510  | 122 38.7436       | 1510        | 0.8                         | 12.2                          | 1.15  | -11.1                                       | 0.8                                 |                 |
| PS00132        | 1   | 8-Jun-11  | 47 16.1952 | 122 35.6142                                   | 47 16.1951  | 122 35.6140       | 1558        | 0.3                         | 4.9                           | 0.53  | -4.4  | 1.4                                 |                 |
|                | 2   |           |            |   | 47 16.1960  | 122 35.6137       | 1608        | 1.6                         | 4.8                           | 0.49  | -4.3  | 1.4                                 |                 |
|                | 3   |           |            |   | 47 16.1955  | 122 35.6128       | 1620        | 1.9                         | 4.2                           | 0.45  | -3.8  | 0.9                                 |                 |
|                | 4   |           |            |   | 47 16.1950  | 122 35.6131       | 1629        | 1.4                         | 4.3                           | 0.42  | -3.9  | 0.9                                 |                 |
| PS00046        | 1   | 9-Jun-11  | 47 11.1750 | 122 39.7494                                   | 47 11.1754  | 122 39.7494       | 0952        | 0.7                         | 82.0                          | 2.06  | -79.9                                       | 1.3                                 |                 |
|                | 2   |           |            |   | 47 11.1757  | 122 39.7492       | 1006        | 1.3                         | 82.1                          | 2.15  | -80.0                                       | 1.2                                 |                 |
|                | 3   |           |            |   | 47 11.1748  | 122 39.7496       | 1020        | 0.4                         | 82.2                          | 2.31  | -79.9                                       | 1.0                                 |                 |
|                | 4   |           |            |   | 47 11.1753  | 122 39.7492       | 1033        | 0.6                         | 82.6                          | 2.39  | -80.2                                       | 1.0                                 |                 |
| PS00238        | 1   | 9-Jun-11  | 47 14.5674 | 122 43.2450                                   | 47 14.5678  | 122 43.2456       | 1129        | 1.1                         | 18.5                          | 2.72  | -15.8                                       | 0.9                                 |                 |
|                | 2   |           |            |   | 47 14.5679  | 122 43.2450       | 1139        | 0.9                         | 18.6                          | 2.76  | -15.8                                       | 0.9                                 |                 |
|                | 3   |           |            |   | 47 14.5676  | 122 43.2460       | 1149        | 1.3                         | 18.4                          | 2.79  | -15.6                                       | 1.3                                 |                 |
| PS00004        | 1   | 9-Jun-11  | 47 21.6036 | 122 40.2720                                   | 47 21.6045  | 122 40.2722       | 1318        | 1.7                         | 32.1                          | 2.60  | -29.5                                       | 1.2                                 |                 |
|                | 2   |           |            |   | 47 21.6045  | 122 40.2715       | 1334        | 1.8                         | 32.1                          | 2.56  | -29.5                                       | 1.1                                 |                 |
|                | 3   |           |            |   | 47 21.6043  | 122 40.2717       | 1346        | 1.4                         | 32.2                          | 2.45  | -29.8                                       | 1.1                                 |                 |
| PS00150        | 1   | 9-Jun-11  | 47 16.8278 | 122 43.4791                                   | 47 16.8278  | 122 43.4791       | 1455        | 0.0                         | 51.2                          | 2.07  | -49.1                                       | 0.8                                 | rocks on Sta.   |
|                | 2   |           |            |   | 47 16.8283  | 122 43.4786       | 1508        | 1.1                         | 51.0                          | 1.96  | -49.0                                       | 0.9                                 | moved target    |
|                | 3   |           |            |   | 47 16.8274  | 122 43.4801       | 1522        | 1.5                         | 51.0                          | 1.85  | -49.2                                       | 0.9                                 | 100 m. SW       |
|                | 4   |           |            |   | 47 16.8276  | 122 43.4793       | 1533        | 0.4                         | 51.0                          | 1.75  | -49.3                                       | 0.9                                 |                 |
| PS00348        | 1   | 9-Jun-11  | 47 16.5604 | 122 40.2666                                   | 47 16.5606  | 122 40.2670       | 1611        | 0.6                         | 7.5                           | 1.40  | -6.1  | 1.1                                 | alternate sta.  |
|                | 2   |           |            |   | 47 16.5602  | 122 40.2664       | 1625        | 0.4                         | 7.0                           | 1.30  | -5.7  | 1.0                                 | station for 260 |
|                | 3   |           |            |   | 47 16.5607  | 122 40.2666       | 1635        | 0.6                         | 7.2                           | 1.23  | -6.0  | 1.0                                 |                 |
|                | 4   |           |            |   | 47 16.5602  | 122 40.2666       | 1647        | 0.4                         | 7.0                           | 1.15  | -5.9  | 1.0                                 |                 |
|                | 5   |           |            |   | 47 16.5602  | 122 40.2666       | 1658        | 0.4                         | 7.2                           | 1.09  | -6.1  | 1.1                                 |                 |
| PS00206        | 1   | 10-Jun-11 | 47 09.1458 | 122 37.3386                                   | 47 09.1459  | 122 37.3385       | 0920        | 0.2                         | 35.3                          | 0.93  | -34.4                                       | 1.6                                 |                 |
|                | 2   |           |            |   | 47 09.1458  | 122 37.3381       | 0929        | 0.6                         | 35.4                          | 1.00  | -34.4                                       | 1.4                                 |                 |
|                | 3   |           |            |   | 47 09.1459  | 122 37.3379       | 0940        | 0.9                         | 35.5                          | 1.09  | -34.4                                       | 1.4                                 |                 |
| PS00374        | 1   | 10-Jun-11 | 47 08.7678 | 122 39.9498                                   | 47 08.7678  | 122 39.9509       | 1012        | 1.4                         | 134.0                         | 1.36  | -132.6                                      | 1.0                                 |                 |
|                | 2   |           |            |   | 47 08.7669  | 122 39.9503       | 1028        | 1.8                         | 134.0                         | 1.51  | -132.5                                      | 1.0                                 |                 |

| Station<br>No. | Rep | Date       | NAI        | n Target<br>D 1983<br>al Minutes<br>Longitude | DGPS L<br>Trimble l<br>(1-m. ac<br>NAD 1983, De<br>Latitude | NT300D<br>curacy) | GPS<br>Time | Dist.<br>to<br>Target<br>m. | Meter<br>Wheel<br>Depth<br>m. | Predicted<br>Tide (m.):<br>Nearest<br>Station | Predicted<br>Mudline<br>Depth, m.<br>(MLLW) | GPS<br>Status<br>HDOP<br><2<br>good | Comments                |
|----------------|-----|------------|------------|---|---|-------------------|-------------|-----------------------------|-------------------------------|---|---|-------------------------------------|-------------------------|
|                | 3   |            | Latitude   | Longitude                                     | 47 08.7677  | 122 39.9514       | 1044        | 2.0                         | 134.3                         | 1.68  | -132.6                                      | 1.0                                 |                         |
| PS00030        | 1   | 10-Jun-11  | 47 09.2394 | 122 40.3224                                   | 47 09.2390  | 122 40.3214       | 1141        | 1.5                         | 52.3                          | 2.33  | -50.0                                       | 1.3                                 |                         |
| 1500050        | 2   | 10 Juli 11 | 47 09.2394 | 122 40.3224                                   | 47 09.2395  | 122 40.3231       | 1151        | 0.9                         | 52.0                          | 2.42  | -49.6                                       | 1.3                                 |                         |
|                | 3   |            |            |   | 47 09.2396  | 122 40.3237       | 1203        | 1.7                         | 51.2                          | 2.52  | -48.7                                       | 1.3                                 |                         |
| PS00388        | 1   | 13-Jun-11  | 47 07.7824 | 122 43.2294                                   | 47 07.7819  | 122 43.2297       | 0939        | 1.0                         | 45.0                          | -0.57   | -45.6                                       | 1.3                                 | alternate sta.          |
|                | 2   |            |            |   | 47 07.7823  | 122 43.2302       | 0949        | 1.0                         | 45.2                          | -0.63   | -45.8                                       | 0.9                                 | station for 270         |
|                | 3   |            |            |   | 47 07.7831  | 122 43.2295       | 0958        | 1.3                         | 45.2                          | -0.66   | -45.9                                       | 0.9                                 |                         |
| PS00044        | 1   | 13-Jun-11  | 47 07.0746 | 122 43.6950                                   | 47 07.0748  | 122 43.6942       | 1029        | 1.1                         | 38.0                          | -0.71   | -38.7                                       | 1.0                                 |                         |
|                | 2   |            |            |   | 47 07.0753  | 122 43.6943       | 1039        | 1.6                         | 37.9                          | -0.69   | -38.6                                       | 1.0                                 |                         |
|                | 3   |            |            |   | 47 07.0747  | 122 43.6959       | 1048        | 1.2                         | 38.0                          | -0.67   | -38.7                                       | 1.0                                 |                         |
| PS00078        | 1   | 13-Jun-11  | 47 09.5682 | 122 44.9808                                   | 47 09.5675  | 122 44.9806       | 1123        | 1.3                         | 56.0                          | -0.49   | -56.5                                       | 0.9                                 |                         |
|                | 2   |            |            |   | 47 09.5681  | 122 44.9811       | 1136        | 0.4                         | 55.8                          | -0.37   | -56.2                                       | 0.9                                 |                         |
|                | 3   |            |            |   | 47 09.5677  | 122 44.9814       | 1147        | 1.2                         | 56.0                          | -0.23   | -56.2                                       | 1.3                                 |                         |
| PS00326        | 1   | 13-Jun-11  | 47 09.9402 | 122 44.6256                                   | 47 09.9411  | 122 44.6251       | 1217        | 1.8                         | 53.4                          | 0.16  | -53.2                                       | 1.4                                 |                         |
|                | 2   |            |            |   | 47 09.9402  | 122 44.6251       | 1228        | 0.6                         | 53.6                          | 0.32  | -53.3                                       | 1.2                                 |                         |
|                | 3   |            |            |   | 47 09.9399  | 122 44.6247       | 1237        | 1.3                         | 53.7                          | 0.48  | -53.2                                       | 1.3                                 |                         |
| PS00086        | 1   | 13-Jun-11  | 47 14.9646 | 122 42.6258                                   | 47 14.9647  | 122 42.6245       | 1351        | 1.7                         | 30.0                          | 1.77  | -28.2                                       | 1.0                                 |                         |
|                | 2   |            |            |   | 47 14.9646  | 122 42.6247       | 1406        | 1.4                         | 30.0                          | 2.02  | -28.0                                       | 1.0                                 |                         |
|                | 3   |            |            |   | 47 14.9648  | 122 42.6237       | 1416        | 2.7                         | 30.0                          | 2.19  | -27.8                                       | 0.9                                 |                         |
| PS00052        | 1   | 13-Jun-11  | 47 10.2360 | 122 46.8306                                   | 47 10.2356  | 122 46.8294       | 1524        | 1.7                         | 109                           | 3.24  | -106  | 0.9                                 |                         |
|                | 2   |            |            |   | 47 10.2361  | 122 46.8293       | 1542        | 1.7                         | 110                           | 3.45  | -107  | 1.1                                 |                         |
|                | 3   |            |            |   | 47 10.2365  | 122 46.8304       | 1557        | 1.0                         | 109                           | 3.59  | -105  | 0.9                                 |                         |
| PS00022        | 1   | 14-Jun-11  | 47 13.5774 | 122 49.0002                                   | 47 13.5772  | 122 49.0013       | 0901        | 1.4                         | 5.8                           | 0.28  | -5.5  | 1.4                                 |                         |
|                | 2   |            |            |   | 47 13.5771  | 122 49.0008       | 0911        | 0.9                         | 5.5                           | 0.12  | -5.4  | 1.4                                 |                         |
|                | 3   |            |            |   | 47 13.5775  | 122 48.9996       | 0923        | 0.8                         | 5.5                           | -0.07   | -5.6  | 2.2                                 |                         |
| PS00054        | 1   | 14-Jun-11  | 47 16.7261 | 122 51.8860                                   | 47 16.7261  | 122 51.8860       | 1007        | 0.0                         | 13.0                          | -0.63   | -13.6                                       | 1.0                                 | moved 200 m<br>offshore |
|                | 2   |            |            |   | 47 16.7264  | 122 51.8854       | 1014        | 0.9                         | 13.0                          | -0.70   | -13.7                                       | 1.0                                 | commercial<br>activity  |
|                | 3   |            |            |   | 47 16.7260  | 122 51.8854       | 1023        | 0.8                         | 12.9                          | -0.75   | -13.7                                       | 1.0                                 |                         |
|                | 4   |            |            |   | 47 16.7259  | 122 51.8857       | 1032        | 0.5                         | 12.9                          | -0.83   | -13.7                                       | 1.0                                 |                         |

| Station<br>No.                   | Rep | Date      | NAI        | n Target<br>D 1983<br>al Minutes<br>Longitude | DGPS L<br>Trimble 1<br>(1-m. ac<br>NAD 1983, De<br>Latitude | NT300D<br>curacy) | GPS<br>Time | Dist.<br>to<br>Target<br>m. | Meter<br>Wheel<br>Depth<br>m. | Predicted<br>Tide (m.):<br>Nearest<br>Station | Predicted<br>Mudline<br>Depth, m.<br>(MLLW) | GPS<br>Status<br>HDOP<br><2<br>good | Comments                |
|----------------------------------|-----|-----------|------------|---|---|-------------------|-------------|-----------------------------|-------------------------------|---|---|-------------------------------------|-------------------------|
| PS00102                          | 1   | 14-Jun-11 | 47 18.2934 | 122 51.3258                                   | 47 18.2930  | 122 51.3256       | 1115        | 0.8                         | 30.0                          | -0.96   | -31.0                                       | 0.9                                 |                         |
|                                  | 2   |           |            |   | 47 18.2930  | 122 51.3250       | 1127        | 1.3                         | 29.8                          | -0.95   | -30.8                                       | 0.9                                 |                         |
|                                  | 3   |           |            |   | 47 18.2938  | 122 51.3242       | 1137        | 2.1                         | 30.0                          | -0.92   | -30.9                                       | 1.3                                 |                         |
| PS00076                          | 1   | 14-Jun-11 | 47 13.5396 | 122 50.4828                                   | 47 13.5396  | 122 50.4826       | 1248        | 0.3                         | 44.5                          | -0.22   | -44.7                                       | 1.3                                 |                         |
|                                  | 2   |           |            |   | 47 13.5397  | 122 50.4829       | 1303        | 0.2                         | 44.8                          | 0.00  | -44.8                                       | 1.2                                 |                         |
|                                  | 3   |           |            |   | 47 13.5398  | 122 50.4827       | 1321        | 0.4                         | 45.2                          | 0.30  | -44.9                                       | 1.3                                 |                         |
| PS00146                          | 1   | 14-Jun-11 | 47 23.3729 | 122 48.9879                                   | 47 23.3729  | 122 48.9879       | 1448        | 0.0                         | 3.2                           | 1.82  | -1.4  | 0.8                                 | moved 300 m<br>offshore |
|                                  | 2   |           |            |   | 47 23.3723  | 122 48.9877       | 1459        | 1.1                         | 3.2                           | 2.05  | -1.2  | 0.9                                 | power lines             |
|                                  | 3   |           |            |   | 47 23.3730  | 122 48.9877       | 1509        | 0.3                         | 3.6                           | 2.28  | -1.3  | 0.8                                 |                         |
| PSNO231                          | 1   | 15-Jun-11 | 47 13.1674 | 123 03.7783                                   | 47 13.1654  | 123 03.7779       | 1002        | 3.7                         | 5.0                           | 1.18  | -3.8  | 1.0                                 |                         |
|                                  | 2   |           |            |   | 47 13.1669  | 123 03.7779       | 1019        | 1.1                         | 4.8                           | 0.88  | -3.9  | 1.0                                 |                         |
|                                  | 3   |           |            |   | 47 13.1660  | 123 03.7787       | 1029        | 2.6                         | 4.4                           | 0.71  | -3.7  | 1.0                                 |                         |
| PSNO227,<br>OB-227S,<br>OB-227SS | 1   | 15-Jun-11 | 47 12.7526 | 123 05.0440                                   | 47 12.7542  | 123 05.0439       | 1107        | 3.0                         | 3.5                           | 0.09  | -3.4  | 0.9                                 |                         |
|                                  | 2   |           |            |   | 47 12.7539  | 123 05.0445       | 1126        | 2.5                         | 3.6                           | -0.19   | -3.8  | 1.3                                 |                         |
|                                  | 3   |           |            |   | 47 12.7521  | 123 05.0435       | 1146        | 1.1                         | 3.0                           | -0.43   | -3.4  | 1.3                                 |                         |
|                                  | 4   |           |            |   | 47 12.7522  | 123 05.0435       | 1156        | 1.0                         | 3.0                           | -0.54   | -3.5  | 1.3                                 |                         |
|                                  | 5   |           |            |   | 47 12.7526  | 123 05.0431       | 1209        | 1.1                         | 2.9                           | -0.66   | -3.6  | 1.4                                 |                         |
| PS00508                          | 1   | 15-Jun-11 | 47 12.7152 | 123 04.7130                                   | 47 12.7152  | 123 04.7138       | 1432        | 1.0                         | 7.0                           | -0.28   | -7.3  | 1.0                                 |                         |
|                                  | 2   |           |            |   | 47 12.7154  | 123 04.7136       | 1449        | 0.8                         | 7.5                           | -0.01   | -7.5  | 0.9                                 |                         |
|                                  | 3   |           |            |   | 47 12.7155  | 123 04.7121       | 1458        | 1.3                         | 7.5                           | 0.14  | -7.4  | 1.6                                 |                         |
|                                  | 4   |           |            |   | 47 12.7154  | 123 04.7142       | 1507        | 1.6                         | 7.8                           | 0.29  | -7.5  | 0.9                                 |                         |
|                                  | 5   |           |            |   | 47 12.7161  | 123 04.7140       | 1518        | 2.1                         | 8.0                           | 0.52  | -7.5  | 0.9                                 |                         |
| PSNO232,<br>OB-232S,<br>OB-232SS | 1   | 15-Jun-11 | 47 13.2406 | 123 03.6894                                   | 47 13.2392  | 123 03.6888       | 1555        | 2.7                         | 3.7                           | 1.26  | -2.4  | 0.9                                 |                         |
|                                  | 2   |           |            |   | 47 13.2410  | 123 03.6908       | 1612        | 1.9                         | 4.0                           | 1.63  | -2.4  | 1.0                                 |                         |
|                                  | 3   |           |            |   | 47 13.2404  | 123 03.6884       | 1633        | 1.3                         | 4.3                           | 2.06  | -2.2  | 1.1                                 |                         |
| PS00164                          | 1   | 15-Jun-11 | 47 13.4990 | 123 03.7176                                   | 47 13.4981  | 123 03.7168       | 1709        | 1.9                         | 6.1                           | 2.80  | -3.3  | 1.0                                 |                         |

| Station<br>No.                               | Rep | Date      | Station Target    NAD 1983    Decimal Minutes    Latitude  Longitude |             | DGPS Location<br>Trimble NT300D<br>(1-m. accuracy)<br>NAD 1983, Decimal Minutes<br>Latitude Longitude |             | GPS<br>Time | Dist.<br>to<br>Target<br>m. | Meter<br>Wheel<br>Depth<br>m. | Predicted<br>Tide (m.):<br>Nearest<br>Station | Predicted<br>Mudline<br>Depth, m.<br>(MLLW) | GPS<br>Status<br>HDOP<br><2<br>good | Comments      |
|--|-----|-----------|--|-------------|---|-------------|-------------|-----------------------------|-------------------------------|---|---|-------------------------------------|---------------|
|  | 2   |           |  |             | 47 13.4992  | 123 03.7176 | 1720        | 0.4                         | 6.6                           | 2.99  | -3.6  | 1.0                                 |               |
|  | 3   |           |  |             | 47 13.5002  | 123 03.7181 | 1733        | 2.3                         | 7.0                           | 3.23  | -3.8  | 1.1                                 |               |
| OB-10S,<br>OB10SS                            | 1   | 15-Jun-11 | 47 14.2560   | 123 02.9760 | 47 14.2568  | 123 02.9768 | 1806        | 1.8                         | 7.0                           | 3.74  | -3.3  | 0.9                                 |               |
| OB-12.5S,<br>OB-12.5SS                       | 1   | 15-Jun-11 | 47 15.0750   | 123 02.1150 | 47 15.0739  | 123 02.1149 | 1832        | 2.0                         | 3.0                           | 4.07  | 1.1   | 0.9                                 |               |
| PS00636,<br>FS00006,<br>OB-636S,<br>OB-636SS | 1   | 16-Jun-11 | 47 12.4548   | 123 05.0148 | 47 12.4549  | 123 05.0147 | 0859        | 0.2                         | 5.3                           | 2.91  | -2.4  | 1.4                                 |               |
|  | 2   |           |  |             | 47 12.4547  | 123 05.0151 | 0909        | 0.4                         | 5.1                           | 2.77  | -2.3  | 1.4                                 |               |
|  | 3   |           |  |             | 47 12.4558  | 123 05.0153 | 0920        | 2.0                         | 5.0                           | 2.59  | -2.4  | 1.4                                 |               |
|  | 4   |           |  |             | 47 12.4554  | 123 05.0144 | 0931        | 1.2                         | 5.0                           | 2.42  | -2.6  | 1.3                                 |               |
|  | 5   |           |  |             | 47 12.4544  | 123 05.0135 | 0942        | 1.8                         | 5.0                           | 2.27  | -2.7  | 1.2                                 |               |
|  | 6   |           |  |             | 47 12.4549  | 123 05.0153 | 0958        | 0.7                         | 4.9                           | 1.99  | -2.9  | 1.0                                 |               |
| PSNO228                                      | 1   | 16-Jun-11 | 47 12.5834   | 123 04.9680 | 47 12.5824  | 123 04.9689 | 1041        | 2.2                         | 3.7                           | 1.26  | -2.4  | 1.6                                 |               |
|  | 2   |           |  |             | 47 12.5830  | 123 04.9691 | 1049        | 1.6                         | 3.7                           | 1.11  | -2.6  | 1.6                                 |               |
|  | 3   |           |  |             | 47 12.5840  | 123 04.9685 | 1058        | 1.3                         | 3.2                           | 0.95  | -2.3  | 0.9                                 |               |
|  | 4   |           |  |             | 47 12.5842  | 123 04.9680 | 1108        | 1.5                         | 3.0                           | 0.79  | -2.2  | 0.9                                 |               |
| PSNO229                                      | 1   | 16-Jun-11 | 47 12.7400   | 123 05.0190 | 47 12.7400  | 123 05.0190 | 1250        | 0.0                         | 2.9                           | -0.61   | -3.5  | 1.6                                 | moved target  |
|  | 2   |           |  |             | 47 12.7399  | 123 05.0183 | 1304        | 0.9                         | 2.7                           | -0.72   | -3.4  | 1.1                                 | 20 m east     |
|  | 3   |           |  |             | 47 12.7396  | 123 05.0194 | 1314        | 0.9                         | 2.3                           | -0.78   | -3.1  | 1.1                                 | shallow water |
| PSNO230                                      | 1   | 16-Jun-11 | 47 12.5267   | 123 04.7564 | 47 12.5274  | 123 04.7566 | 1344        | 1.3                         | 4.9                           | -0.88   | -5.8  | 0.8                                 |               |
|  | 2   |           |  |             | 47 12.5270  | 123 04.7558 | 1355        | 0.9                         | 5.0                           | -0.89   | -5.9  | 0.8                                 |               |
|  | 3   |           |  |             | 47 12.5274  | 123 04.7561 | 1406        | 1.4                         | 5.0                           | -0.88   | -5.9  | 0.9                                 |               |
|  | 4   |           |  |             | 47 12.5265  | 123 04.7565 | 1416        | 0.4                         | 4.8                           | -0.86   | -5.7  | 0.8                                 |               |
|  | 5   |           |  |             | 47 12.5262  | 123 04.7563 | 1430        | 0.9                         | 4.6                           | -0.76   | -5.4  | 0.9                                 |               |
| PSNO248                                      | 1   | 17-Jun-11 | 47 08.5717   | 122 50.1324 | 47 08.5718  | 122 50.1315 | 0951        | 1.2                         | 8.7                           | 1.86  | -6.8  | 1.0                                 |               |
|  | 2   |           |  |             | 47 08.5720  | 122 50.1328 | 1009        | 0.8                         | 8.5                           | 1.56  | -6.9  | 1.0                                 |               |
|  | 3   |           |  |             | 47 08.5717  | 122 50.1343 | 1018        | 2.4                         | 8.1                           | 1.39  | -6.7  | 1.0                                 |               |

| Station<br>No. | Rep | Date      | Station Target    NAD 1983    Decimal Minutes    Latitude  Longitude |             | DGPS Location<br>Trimble NT300D<br>(1-m. accuracy)<br>NAD 1983, Decimal Minutes<br>Latitude Longitude |             | GPS<br>Time | Dist.<br>to<br>Target<br>m. | Meter<br>Wheel<br>Depth<br>m. | Predicted<br>Tide (m.):<br>Nearest<br>Station | Predicted<br>Mudline<br>Depth, m.<br>(MLLW) | GPS<br>Status<br>HDOP<br><2<br>good | Comments |
|----------------|-----|-----------|--|-------------|---|-------------|-------------|-----------------------------|-------------------------------|---|---|-------------------------------------|----------|
|                | 4   |           |  | 6           | 47 08.5724  | 122 50.1315 | 1031        | 1.7                         | 8.0                           | 1.15  | -6.9  | 1.3                                 |          |
| PSNO249        | 1   | 17-Jun-11 | 47 08.1040   | 122 50.1473 | 47 08.1035  | 122 50.1476 | 1106        | 1.0                         | 5.8                           | 0.54  | -5.3  | 0.9                                 |          |
|                | 2   |           |  |             | 47 08.1040  | 122 50.1473 | 1119        | 0.0                         | 5.6                           | 0.33  | -5.3  | 1.3                                 |          |
|                | 3   |           |  |             | 47 08.1046  | 122 50.1472 | 1134        | 1.1                         | 5.2                           | 0.08  | -5.1  | 1.3                                 |          |
| PSNO250        | 1   | 17-Jun-11 | 47 08.6698   | 122 50.4779 | 47 08.6697  | 122 50.4784 | 1259        | 0.7                         | 5.0                           | -0.83   | -5.8  | 1.2                                 |          |
|                | 2   |           |  |             | 47 08.6703  | 122 50.4779 | 1310        | 0.9                         | 5.0                           | -0.86   | -5.9  | 1.2                                 |          |
|                | 3   |           |  |             | 47 08.6704  | 122 50.4774 | 1320        | 1.3                         | 5.1                           | -0.89   | -6.0  | 1.2                                 |          |
| PSNO234        | 1   | 20-Jun-11 | 47 06.9598   | 123 02.3086 | 47 06.9601  | 123 02.3088 | 1020        | 0.6                         | 4.9                           | 3.22  | -1.7  | 1.3                                 |          |
|                | 2   |           |  |             | 47 06.9601  | 123 02.3089 | 1030        | 0.7                         | 5.0                           | 3.16  | -1.8  | 1.3                                 |          |
|                | 3   |           |  |             | 47 06.9593  | 123 02.3076 | 1039        | 1.6                         | 5.0                           | 3.09  | -1.9  | 1.3                                 |          |
| PSNO233        | 1   | 20-Jun-11 | 47 09.3178   | 123 00.2629 | 47 09.3174  | 123 00.2620 | 1130        | 1.4                         | 8.4                           | 1.90  | -6.5  | 1.4                                 |          |
|                | 2   |           |  |             | 47 09.3184  | 123 00.2638 | 1141        | 1.6                         | 8.0                           | 1.81  | -6.2  | 1.4                                 |          |
|                | 3   |           |  |             | 47 09.3177  | 123 00.2623 | 1152        | 0.8                         | 8.0                           | 1.72  | -6.3  | 1.1                                 |          |
| PS00036        | 1   | 20-Jun-11 | 47 08.1894   | 123 01.0086 | 47 08.1896  | 123 01.0080 | 1320        | 0.8                         | 11.3                          | 0.83  | -10.5                                       | 1.1                                 |          |
|                | 2   |           |  |             | 47 08.1893  | 123 01.0088 | 1328        | 0.3                         | 11.0                          | 0.75  | -10.3                                       | 1.1                                 |          |
|                | 3   |           |  |             | 47 08.1896  | 123 01.0079 | 1337        | 1.0                         | 11.0                          | 0.66  | -10.3                                       | 1.0                                 |          |
| PSNO235        | 1   | 20-Jun-11 | 47 09.1915   | 122 58.6520 | 47 09.1914  | 122 58.6519 | 1404        | 0.2                         | 9.0                           | 0.42  | -8.6  | 0.9                                 |          |
|                | 2   |           |  |             | 47 09.1922  | 122 58.6514 | 1418        | 1.5                         | 8.9                           | 0.31  | -8.6  | 1.0                                 |          |
|                | 3   |           |  |             | 47 09.1921  | 122 58.6524 | 1430        | 1.2                         | 8.8                           | 0.23  | -8.6  | 0.9                                 |          |
| PS00204        | 1   | 20-Jun-11 | 47 12.2340   | 122 54.1416 | 47 12.2336  | 122 54.1417 | 1542        | 0.8                         | 5.0                           | 0.01  | -5.0  | 1.0                                 |          |
|                |     |           |  |             | 47 12.2344  | 122 54.1418 | 1554        | 0.8                         | 5.0                           | 0.01  | -5.0  | 1.0                                 |          |
|                |     |           |  |             | 47 12.2349  | 122 54.1407 | 1604        | 2.0                         | 5.0                           | 0.04  | -5.0  | 1.0                                 |          |
| PSNO240        | 1   | 21-Jun-11 | 47 05.9624   | 122 58.8208 | 47 05.9624  | 122 58.8212 | 0938        | 0.5                         | 8.0                           | 3.15  | -4.9  | 1.0                                 |          |
|                |     |           |  |             | 47 05.9623  | 122 58.8198 | 0951        | 1.3                         | 8.0                           | 3.16  | -4.8  | 1.0                                 |          |
|                |     |           |  |             | 47 05.9623  | 122 58.8217 | 1001        | 1.2                         | 8.0                           | 3.15  | -4.9  | 1.0                                 |          |
| PSNO238        | 1   | 21-Jun-11 | 47 06.7964   | 122 57.4226 | 47 06.7967  | 122 57.4227 | 1027        | 0.6                         | 14.0                          | 3.07  | -10.9                                       | 1.3                                 |          |
|                |     |           |  |             | 47 06.7963  | 122 57.4231 | 1039        | 0.7                         | 14.0                          | 3.03  | -11.0                                       | 1.2                                 |          |
|                |     |           |  |             | 47 06.7967  | 122 57.4217 | 1050        | 1.3                         | 13.8                          | 2.99  | -10.8                                       | 0.9                                 |          |
| PSNO239        | 1   | 21-Jun-11 | 47 07.3304   | 122 58.6253 | 47 07.3302  | 122 58.6245 | 1125        | 1.1                         | 12.9                          | 2.79  | -10.1                                       | 1.4                                 |          |
|                | 2   |           |  |             | 47 07.3305  | 122 58.6247 | 1137        | 0.8                         | 12.9                          | 2.70  | -10.2                                       | 1.4                                 |          |

| Station<br>No.    | Rep | Date      | NAI        | n Target<br>D 1983<br>al Minutes<br>Longitude | DGPS L<br>Trimble M<br>(1-m. ac<br>NAD 1983, De<br>Latitude | NT300D<br>curacy) | GPS<br>Time | Dist.<br>to<br>Target<br>m. | Meter<br>Wheel<br>Depth<br>m. | Predicted<br>Tide (m.):<br>Nearest<br>Station | Predicted<br>Mudline<br>Depth, m.<br>(MLLW) | GPS<br>Status<br>HDOP<br><2<br>good | Comments                |
|-------------------|-----|-----------|------------|---|---|-------------------|-------------|-----------------------------|-------------------------------|---|---|-------------------------------------|-------------------------|
|                   | 3   |           |            |   | 47 07.3310  | 122 58.6254       | 1149        | 1.1                         | 12.9                          | 2.62  | -10.3                                       | 1.2                                 |                         |
| PSUW116<br>BI-116 | 1   | 21-Jun-11 | 47 07.8762 | 122 54.6552                                   | 47 07.8764  | 122 54.6553       | 1301        | 0.4                         | 18.0                          | 1.94  | -16.1                                       | 1.2                                 |                         |
|                   | 2   |           |            |   | 47 07.8759  | 122 54.6559       | 1314        | 1.0                         | 17.8                          | 1.82  | -16.0                                       | 1.2                                 |                         |
|                   | 3   |           |            |   | 47 07.8762  | 122 54.6549       | 1325        | 0.4                         | 18.0                          | 1.70  | -16.3                                       | 0.8                                 |                         |
| PSUW244           | 1   | 21-Jun-11 | 47 08.6899 | 122 55.1094                                   | 47 08.6899  | 122 55.1094       | 1450        | 0.0                         | 31.0                          | 0.86  | -30.1                                       | 0.9                                 | moved 200 m<br>offshore |
|                   | 2   |           |            |   | 47 08.6903  | 122 55.1097       | 1502        | 0.8                         | 31.0                          | 0.76  | -30.2                                       | 1.1                                 | rocks & gravel          |
|                   | 3   |           |            |   | 47 08.6899  | 122 55.1096       | 1512        | 0.3                         | 30.9                          | 0.69  | -30.2                                       | 1.4                                 |                         |
|                   | 4   |           |            |   | 47 08.6903  | 122 55.1096       | 1522        | 0.8                         | 31.0                          | 0.62  | -30.4                                       | 1.4                                 |                         |
|                   | 5   |           |            |   | 47 08.6892  | 122 55.1093       | 1532        | 1.3                         | 30.9                          | 0.57  | -30.3                                       | 1.4                                 |                         |

## Appendix C. DNA Barcoding Project Proposal

### **Evaluation of DNA Barcoding as a Tool for Assessing Marine Macrobenthic Biological Communities**

#### David Gillette, Southern California Coastal Water Research Project

Measures of macrobenthic community structure are well established tools for assessing the habitat quality of marine ecosystems around the World. These methods involve assigning indices based on the species composition and abundance that are used to rank the relative quality of sites along gradients of disturbance. Although well validated, one of the challenges of this approach is the time associated with identification of several hundred specimens per site. Furthermore, limitation of our current taxonomy may only allow identification of some species to genus or complex level, thereby influencing the resolution of the biological indices uses to assess condition. There are a variety of genetic-based approaches to evaluating macrobenthic community structure (collectively referred to as a DNA barcoding) that may potentially increase the resolution of our taxonomic analysis and reduce the cost and time to process samples for environmental monitoring and assessment. The DNA barcoding process yields unique taxonomic units analogous to species based upon the degree of dissimilarity in selected DNA basepairs among the organisms of interest. Before techniques can be developed for measuring community structure with DNA barcodes, the barcoding approach must be tested against the current assessment methodology standards of precision and accuracy. The goal of this project is to begin assessing the utility of this genetic-based approach by comparing it to the presently used morphometric character-based identifications in order to address questions of taxonomic resolution and population heterogeneity.

# Question 1 – Ability of DNA barcoding to discern potential differences in populations of cosmopolitan species

Populations of common species may vary along spatial gradients due to processes such as genetic drift, founder effects, or bottle necks. These differences have the potential to influence conclusions about environmental condition based on benthic indices. A potential application of DNA barcodes is to assess the effect of spatial gradients on the genetic structure of populations

of commonly occurring, cosmopolitan species that are currently considered the same based on morphometric structure and ecology/life history. This analysis will investigate the ability of DNA barcoding to discern spatial differences in populations of single species along a gradient from Puget Sound, Washington to San Diego, California, and region that includes several potential biogeographic breaks.

After discussion with the marine benthic barcoding workgroup the following species will be targeted for collection and analysis [(B) denotes brooding taxa and (P) denotes pelagic broadcasting taxa]: Ampelisca careyi (B), A. agassizi, Euphilomedes carcharodonta (B), Nephtys caecoides (P), N. ferruginea (P), Spiophanes berkleyorum (P), S. norrisi (P), and Tellina *modesta* (P). Some organisms have already been collected by SCCWRP's partners – San Diego County Sanitation District, Orange County Sanitation District, Los Angeles County Sanitation District, San Francisco Estuarine Institute, and Washington State Department of Ecology. Where spatial gaps exist for different species, additional material will be collected in the course of other monitoring projects. The identity of each species to be used in this study is not in dispute among expert taxonomists and all are commonly observed along the entire coastline. Target species have also been selected to encompass disparate reproductive strategies: those species that brood their young vs. those that broadcast pelagic larvae. These selections were made as "best" and "worst-case" scenarios for population genetic structure and sensitivity of the barcoding process to evaluate individuals of the same species as different taxonomic units. Those species that brood their young will have greater likelihood of having differences in the genetic structure within populations of the same species, while those broadcast spawning species will have less population-level genetic structure.

Variation in DNA barcode derived genetic information across all of the geographic locals will be compared within each species and, where available, to other genetic identification techniques that are known to have greater or lesser sensitivity to natural genetic drift (e.g., ribosomal DNA, microsatellites, or whole genome). The results of this work will help to inform our larger goal of developing DNA barcode-based assessment tools for the marine environment by beginning to document the variance and sensitivity of this molecular-based identification approach compared to traditional taxonomy.

# Question 2 – Utility of DNA barcoding to improve taxonomic resolution of difficult to identify species.

The resolution of current benthic indices may be improved by augmenting our ability to identify specimens that can only now be identified to genus or complex due to their small size, fragility, or phenotypic plasticity. This analysis will investigate the use of genetic information in concert with morphological characters to better understand the composition of marine macrobenthic communities. A set of target complexes have been identified based on their abundance, taxonomic ambiguity, interest of local taxonomists, importance to calculation of the CA benthic response index (BRI). To the extent that they can be collected, these specimens will be identified to the lowest taxonomic level commonly applied, vouchered, and analyzed for their DNA barcodes.

After discussion with the marine benthic barcoding workgroup the following taxa will be targeted for collection and analysis: *Aphelochaeta glandaria* complex, *Capitella capitata* complex, *Leptochelia dubia*, *Pholoe* spp., *Protomedia* spp., *Scolopolus arminger*, *Spio filicornus*, and *Tellina* spp. Some organisms have already been collected by SCCWRP's partners – San Diego County Sanitation District, Orange County Sanitation District, Los Angeles County Sanitation District, San Francisco Estuarine Institute, and Washington State Department of Ecology. Where spatial gaps exist for different species, additional material will be collected in the course of other monitoring projects.

Species composition based on genetic analysis will be compared to that obtained by traditional morphology-based methods to determine how DNA barcoding affects conclusions regarding environmental condition. The separation of individuals from the same complex based upon their DNA barcode will be used by taxonomists, where appropriate, to inform morphologically-based dichotomous keys and the eventual construction of new monophyletic species from formerly polyphyletic taxa/species complexes. This information can then be used to further refine current assessment tools based upon macrobenthic community structure, as well as help to determine the utility of DNA barcode-based assessment tools for the marine environment.

# Appendix D. Protocols for collection and preservation of, and tissue preparation for DNA barcoding of marine benthic invertebrates.

## Protocols for the Collection of Benthic Infaunal Invertebrate Samples for Bar Code Processing

(after D. Steinke, as interpreted by M. Dutch, 3/25/2010; updated 8/16/2011)

### **Collection of Benthic Infaunal Invertebrate Samples:**

- Collect bottom sediment samples with a double vanVeen grab.
- Place grab samples on screen with 1mm mesh, and gently rinse sediment through the screen with ambient seawater.
- Collect all organisms and sediment retained on the screen and place in collection container (Ziplock freezer bag, jar, etc).
- Fill collection container with 95% ethanol to five times the volume of the sample.
- Bring samples back to Ecology HQ and place in walk-in cooler.
- Exchange the ethanol in the sample bags one time within 24-48 hours (preferably within 24 hours), retaining the 5:1 ratio of 95% ethanol:sample. Add glycerin (5% per total volume) to each container to preserve suppleness of the specimens.
- Sample holding times in ETOH:
  - $\circ$  Room temperature 2-3 months
  - $\circ$  Refrigerated (4°C) 1 year
  - Freeze  $(-20^{\circ}C)$  many years

### \*\* Formalin must be strictly avoided at all steps of sample processing! Sample Sorting and Taxonomy:

- Sort samples into major taxa groups (Annelids, Molluscs, Arthropods, Echinoderms, Miscellaneous Taxa) in vials filled with 95% ETOH. Maintain the 5:1 ratio of 95% ETOH:sample in these vials. Return samples to refrigeration.
- Specialized taxonomists to identify organisms in each major taxa group, retaining 10 specimens of each in 95% ETOH for barcoding analysis. Return specimens to refrigeration.
- Extra specimens (>10), can be fixed in formalin and archived.

### Subsampling for Barcoding and Collection of Metadata:

- Obtain 96-well microtiter plates from Canadian Centre for DNA Barcoding (CCDB)
- Follow instructions received with plates to collect and retain tissue samples from each ETOH-preserved specimen in the plate wells.
- There is one control well, which leaves 95 wells for specimen samples. The amount of tissue required shouldn't be more than a match head in size.
- Very small specimens can be sent whole in a well. Barcoding process does not consume the sample, and the specimen can be returned for vouchering.

- Metadata collection: CCDB to provide data spreadsheets for metadata of specimens as well as plate records that connect metadata with sample position on the plate. Specimen metadata will have to go on the BOLD database first (<u>www.boldsystems.org</u>) before they can enter the samples in their lab system. Sequences, trace files etc. will be uploaded to the database. There is also an option to upload images to the database (if you do images of your specimens).
- Plates are then shipped to CCDB in Guelph, Ontario. Wells contain ETOH. If this poses a problem for shipping, ETOH may be evaporated prior to shipping, if shipping time is relatively short.
- Tissue samples undergo barcoding at CCDB, and data are released as soon as possible.
- CCDB has the means to send some of their students/personnel to come to your facility to do, or assist, with the subsampling process. Contact Dr. Peter Miller (<u>pemiller@uguelph.ca</u>)or Dirk Steinke (<u>dsteinke@uoguelph.ca</u>) to make these arrangements.
- When barcoding is complete, specimens may be transferred to formalin for fixation and long-term archives.

## Equipment list – to preserve 10 samples:

20 - <sup>1</sup>/<sub>2</sub> gallon or 1 gallon jars for samples 10 gallons 95% ETOH Coolers/buckets to store samples in

### CCDB INTERNATIONAL BARCODE OF LIFE PROJECT MICROPLATE AND DATA SUBMISSION PACKAGE



Column Markers

This Submission Package is aimed to facilitate the exchange of tissue samples and specimen data between Research Collaborators and the Canadian Centre for DNA Barcoding (CCDB), one of the central analytical nodes for the International Barcode of Life Project (iBOL). It contains microplates for housing tissue samples that should be returned to the Biodiversity Institute of Ontario (the hosting institution of the CCDB) for analysis and spreadsheets for entering specimen data for submission to the Barcode of Life Data Systems (BOLD).

#### MICROPLATE

Each microplate contains sampling wells that are arranged in a 12×8 format. The sampling array starts with well A01. Well H12 should be left empty for control, so each plate will accommodate 95 samples. See below for details of the sampling procedure.

Each plate will be individually numbered, and will be shipped to you with the label pre-affixed to the plate. Each label contains a unique barcode and human-readable identifier (CCDB Number). The CCDB number should be provided in the corresponding *CCDB Record* spreadsheet (see last page).

and will be shipped plate. Each label eadable identifier buld be provided in eet (see last page). e top of the zip-lock information: illing sample wells Microplate label (on side of plate) (CCDB-00001

A separate large label will be affixed to the top of the zip-lock bag containing the plates, with the following information: Sent to: *Collaborator* responsible for filling sample wells and providing specimen data (tissue provider).

Sent by: CCDB contact responsible for the shipment (recipient). This person will usually oversee analyses and facilitate the submission of specimen data to BOLD; may coincide with BOLD Project Manager.

Note: Before adding samples into a plate, make sure the label is attached to the side corresponding to row H. Always work with the plate label facing towards you. Pay special attention to the position of row (A through H) and column (1 through 12) markers: they should be on the left and top margins of the plate, respectively.

Note: As of June 2008, the sampling order and procedure for microplates and the number and position of control wells has been altered. Collaborators are strongly encouraged to read these instructions carefully and to follow them.

#### DATA SUBMISSION SPREADSHEETS

The CD included in this Sample Submission Package contains three blank spreadsheets corresponding to the three blocks of data needed for a complete specimen record (plate record, specimen data and image data) and a set of help files.

- The file CCDB-00000\_Record.xls is intended to record locations of samples in the corresponding microplate (or array of plates) for the lab staff running the molecular analyses. Therefore it is a critical component of the analytical chain. Each sample must be assigned a Sample ID — a *unique individual identifier unambiguously linking the tissue sample with its source specimen* (ideally, a permanent collection catalogue number prefixed by the museum acronym or, if unavailable, a field collection number prefixed by the collector's initials). Note: Note: A single CCDB Record can contain data for up to 10 boxes. See last page for details.
- The file SpecimenData.xls is intended for entering geographic, taxonomic and other collection data for the specimens to be analyzed. The 'Sample ID' field should contain numbers identical to those entered in the Plate
- specimens to be analyzed. The 'Sample ID' field should contain numbers identical to those entered in the Plate Record. Please provide as many details for each entry as possible. Refer to the help file DataFormat.pdf for further information on filling in this sheet.
- The file ImageData.xIs in the folder /ImageSubmission/ should house data on the digital images of the voucher specimens that provided the tissue samples. Refer to the help file ImageSubmission.pdf for details on the image submission procedure.

NOTE: Submission of specimen data and images is independent from sample submission. Submission of the specimen data and images to BOLD is a critical prerequisite before tissue samples can be analyzed in the lab. To facilitate effective processing of samples, their accompanying data must be submitted in a BOLD compliant format. To begin the sampling process, position the plate on a flat surface with the plate label facing towards you.

The column markers (1–12) should be at the top and the row markers (A–H) should be on the left side.



or





If samples are prone to spontaneous displacement because of static electricity (e.g., dry insect legs), sampling wells should be pre-filled with 30  $\mu$ l (microlitres) of 95% Ethanol, e.g., using a multi channel pipettor. If a pipettor is not available, add one drop of Ethanol to each well using an eyedropper, just prior to sampling.

Note: Do not add excess ethanol - this may cause well caps to pop off during shipping. If the samples are compact and were previously fixed in ethanol (e.g. vertebrate muscle tissue), then no fixative should be added to the plate. Tissue that has not been dried or preserved should not be sampled into a microplate. Never use ethanol if tissue was previously fixed with Dimethyl sulfoxide (DMSO)



Before proceeding with sampling, place the cap strips (supplied with the sampling kit) over all well rows to avoid cross-contamination during sampling. Observe the orientation of cap strips: terminal markers "1" (wide) and "12" (narrow); these should match the corresponding columns of the plate. Do not fasten caps tightly, as you will need to remove the strips just prior to sampling.

When sampling, remove corresponding cap strips one at a time and fasten them back when paused or after finishing each row.



Start the sampling process with A01 (row 1) and proceed in alphanumerical order to A12 (left to right). When done with the first row, proceed to the second row (B01) and repeat the process until all 12 rows are filled. Do not leave empty wells in the middle of the plate. While sampling, remove only one cap strip at a time to prevent crosscontamination.

IMPORTANT: Do not fill the last well, H12! It should be left empty as a negative control.

As you proceed with sampling, keep a full record of Sample ID's in the Data Input worksheet of the corresponding CCDB RECORD workbook. For details, refer to instructions on page 5 of this manual and in the CCDB Record Data Input Sheet.

#### SAMPLING PROCESS: THE PROCEDURE



Before beginning the sampling procedure, remove the cap strip from the first row. If required, make sure that wells are pre-filled with fixative (see previous page). Sample or subsample the right amount of tissue with fine forceps (as shown in the image) and place it into the sampling well. Confirm that the tissue remains inside the well. Once done, enter the corresponding sample ID into the CCDB Record (see below).

Note: DO NOT place any foreign objects (e.g. labels) into sampling wells.

Before proceeding to the next sample, ensure that no residual tissue is present on the forceps by rinsing them in 95% Ethanol and wiping them with a clean napkin or paper towel.

When the work environment permits, use flame (e.g., for dry insects) or bleach/specialized detergent such as Eliminase (e.g., for vertebrate tissue) to sterilize your sampling tools.





Note: If using bleach or detergent, make sure that all chemicals are completely removed from the tools by thoroughly rinsing them in distilled water before the next sampling round, to avoid DNA degradation.

Below are some examples of recommended tissue sizes for sampling into microplates:



- Small insect: whole leg, antenna ca. 5–6 mm length
  Large insect: femur only ca. 2–4 mm length
- Vertebrate/invertebrate: muscle ca. 8 mm<sup>3</sup> volume or 2 mm diameter
- 2-dimensional tissue: skin/body wall ca. 3-4 mm diameter
- Minute invertebrate: whole specimen ca. <3 mm length



Note: Do not place excessive tissue into the sampling wells - this may inhibit DNA extraction. If the sample exceeds the recommended dimensions, subdivide it into fragments to obtain the right amount.

Avoid sampling from body parts containing scales, hairs or bristles, when possible. Avoid sampling from digestive tracts or from areas which may have been in contact with digestive tract contents.

To visualise well contents (e.g., to evaluate the correct amount of fixative or tissue sampled) examine the plate from below.

After samples have been added to all wells in a row, replace the cap strip and seal it firmly before proceeding with next row.

When sampling into the last row (Row H), remember to leave the last well (H12) empty. It is OK to add fixative to this well if dispensing with a multi-channel pipettor.

Once the plate is filled with samples, ensure that all cap strips are pressed firmly into the wells.



Note: All samples sent in microplates will be completely used up for molecular analysis; no residual tissue will remain.

Open the blank file titled CCDB-00000\_Record.xls and follow instructions typed in green in the grey field of the worksheet titled "DATA INPUT".

1. Select the type of sample medium from the dropdown menu. It should be "microplate".

If intending to fill a multiple plate array, mark the checkbox "Multiple array..." in the top right of the sheet.

Enter the CCDB number(s) in the designated field(s) (type in digits only, do not add prefixes) - this will unhide the fields for entering Sample ID numbers.

4. After filling each sampling well, enter the Sample ID number into the corresponding cell of the CCDB Record DATA INPUT sheet. Ultimately, each CCDB Record should contain 95 entries per plate, corresponding to 95 samples. If preferred, the entire spreadsheet could be populated at once (e.g., by pasting a column of data), provided that all measures are taken to ensure complete correspondence between samples and CCDB Record.

|    | A                                | в                                     | С             | D         | ε                  | F                             | G                      | н                    | 1               |
|----|----------------------------------|---------------------------------------|---------------|-----------|--------------------|-------------------------------|------------------------|----------------------|-----------------|
|    | >                                | Canadian Cer                          | tre for DNA E | Barcoding | y .                | Select type of se             | moling medium          | microplete           |                 |
|    | SCCDB                            | SAMPLE REC                            | ORD DATA I    | VPUT SH   | EET                |                               |                        |                      |                 |
|    | 10000                            | SPEED ALL FILLS                       |               |           |                    | P Nubple                      | array (up to 10 tube r | ecks, micropietes or | blotting cantel |
| 1  | Sample Locator                   | Sample ID                             |               |           |                    |                               | CCDB Number            |                      |                 |
| 2  | CC08-00001 A01                   | SAMPLE-0001                           |               | En        | ter columnar       | array of CCDB numbers:        | CCDB-00001             |                      |                 |
| 3  | CC00-0001 A02                    | SAMPLE-0002                           |               |           | in numerical       | order (up to 10 numbers)      | CCDB-00002             |                      |                 |
| 4  | CCD8-00001 ACG                   | SAMPLE-0003                           |               |           |                    | next cell will appear after   | CCDB-00003             |                      |                 |
| 5  | CCDB-00001 A04                   | SAMPLE-0004                           |               |           |                    | you fill the previous one     |                        |                      |                 |
| 6  | CC08-0001 A05                    |                                       |               |           |                    |                               | CCDB-00005             |                      |                 |
| 7  | CCDB-00001 A06                   |                                       |               |           | NOTE: Use          | the same type of medium       | CCDB-00006             |                      |                 |
| 8  | CCDB-00001 A07                   |                                       |               |           |                    | throughout the array          | CCDB-00007             |                      |                 |
| 9  | CCD8-00001 A08                   |                                       |               |           |                    |                               |                        |                      |                 |
| 10 | CC08-0001 A09                    | · · · · · · · · · · · · · · · · · · · |               |           |                    | le ID's for your samples      |                        |                      |                 |
| 11 | CCD8-00001 A10                   |                                       |               | into th   | e white cells (    | on the left (column B)        |                        |                      |                 |
| 12 | CC08-0001 A11                    |                                       |               |           |                    |                               |                        |                      |                 |
| 13 | CCD8-00001 A12                   |                                       |               | NOTE:     | Do not enter       | data for control tube H12     |                        |                      |                 |
|    | CCDB-00001 B01                   |                                       |               |           |                    |                               |                        |                      |                 |
|    |                                  |                                       | -             |           |                    | ta entry, rename this file t  |                        |                      | _               |
|    |                                  |                                       | -             | CCDB      | number(s) as       | shown here:                   | CCDB-00001-00          | 007_Record.sl        | •               |
|    | CC08-0001 804                    |                                       | -             |           |                    |                               |                        |                      |                 |
|    | CC08-0001 805                    |                                       | -             |           |                    |                               |                        |                      |                 |
|    | GG08-0001 005                    |                                       | -             | 1000      |                    |                               |                        |                      |                 |
|    | GC08-0001 807                    |                                       | -             | Please    | ensure that        | t your data submission        | meets the foll         | owing criteria       | <u>n</u>        |
|    | CC08-00001 B08                   |                                       | -             |           |                    |                               |                        |                      |                 |
|    | CCD8-0001 809                    |                                       | -             |           |                    | ue identifiers unambiguo      |                        |                      |                 |
|    |                                  |                                       | -             | with th   | e correspond       | ing BOLD record and col       | lection voucher        | specimen             |                 |
|    | CCD8-00001 811                   |                                       | -             | -         |                    |                               |                        | A                    | 000480          |
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| 20 | CGDB-00001 C01                   |                                       | -             |           |                    | of the corresponding tube     |                        | 12222222             | 22222           |
|    | GG08-00001 G02                   |                                       |               |           |                    | r to sample layout map or     |                        | 100000000            |                 |
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| 28 | CC06-0001 C04<br>CC08-0001 C05   |                                       |               |           | -                  |                               |                        |                      |                 |
| 30 |                                  |                                       | -             |           |                    | AMPLE RECORD should           |                        |                      |                 |
|    | CC08-00001 C05                   |                                       |               | in the l  | espective ap       | ECINEN DATA RECORD            | or submission in       | NO BOLD              |                 |
|    | CCD8-00001-007<br>CCD8-00001-008 |                                       | -             | Quer - I  | The shares         | ha at least 2 (thread) at any | stem long              |                      |                 |
| 33 |                                  | Submission localts / Far              |               | a sample  | Dissions           | be at least 3 (three) chara   | cters long             |                      |                 |

Sample ID's should be entered in columnar format in the white cells of the DATA INPUT spreadsheet. Please ensure that the Locator next to each record matches the position of the corresponding sampling well. Do not enter data for the control well (H12).

Make sure that your data submission adheres to the requirements outlined in the 'DATA INPUT' worksheet. Watch for error messages appearing in red colour on yellow background in the field to the right of the corresponding CCDB numbers and Sample ID records and change your entries accordingly.

To visualize correspondence between the data recorded and the position of samples in the microplate, refer to the next worksheet titled 'Submission Results'. If errors were detected when entering Sample ID information, an additional map will be displayed below the general layout map to help localize problematic sample entries. Please ensure that all error messages disappear before submitting the CCDB Record.

When data entry is completed, rename the file to incorporate the CCDB numbers included in it, for example, rename it to CCDB-00001\_Record.xls' for a single plate or to 'CCDB-00001\_00007\_Record.xls' for a multiple plate array.

NOTE: All coloured cells in the CCDB Record workbook are write-protected to secure formulas and cross-links. Please type/paste your data only into white cells. Avoid moving (cutting and pasting) data between cells; use the copy-paste-delete procedure instead.

4

IMPORTANT: Fill all 95 sampling wells in each plate before proceeding to the next plate. Do not ship back partly filled plates, unless specifically arranged with your BOLD Project Manager. Whenever a plate is transferred to another person for tissue sampling, please notify your BOLD Project Manager.

After you have completed the sampling procedure, please return your plates by courier or registered mail to the following address. Please indicate a nil value on the shipping invoice.

Sample Submission University of Guelph Biodiversity Institute of Ontario 50 Stone Road East Guelph, Ontario, Canada N1G 2W1 Phone: +1 (519) 824-4120 ext. 56393

NOTICE: Unless explicitly negotiated otherwise, all biological materials shipped to the Biodiversity Institute of Ontario fall under the standard provisions of the BIO Tissue Policy and BIO Biological Material Transfer Agreement. These documents can be downloaded from the CCDB website (<u>www.dnabarcoding.ca</u>) or obtained, upon request, from your BOLD Project Manager or from the BIO curator of zoological collections <<u>aborisen@uoguelph.ca></u>. A printed version of the Biological Material Transfer Agreement and two hard copies of the Implementing Letter signed by a CCDB representative (Recipient Scientist) should have been sent to you with the first sampling kit. Please sign one copy of the BMTA and return it with the first batch of samples.

DISCLAIMER: It is the sender's responsibility to ensure that biological materials are shipped to the Biodiversity Institute of Ontario in compliance with any applicable shipping regulations, that they have been obtained under appropriate collection and animal care permits in their country of origin and that the necessary export/import documentation required by Canadian and International customs and conservation authorities has been provided, including, but not limited to:

- a) Export permit and/or zoosanitary certificate from the country of origin (if applicable);
- b) CITES registry certificate for the provider institution (if applicable);
- c) Canadian Food Inspection Agency import permit (if applicable).

The Biodiversity Institute of Ontario cannot be held responsible in the event the provider fails to supply proper shipping documentation, causing the shipment to be held up in customs, or any penalties resulting thereof. Upon request, BIO staff will advise on Canadian import requirements and assist in obtaining relevant import permits.

The Biodiversity Institute of Ontario is a CITES-registered institution (registry certificate CA022).

#### SUBMITTING DATA

CCDB Record files should be e-mailed to the lab manager at the Canadian Centre for DNA Barcoding <conchris@uoguelph.ca>, with a copy to your Project Contact or shipped on the CD together with the filled plates.

The Specimen Data Record file should be sent by e-mail to the BOLD team <mmilton@uoguelph.ca> with a copy to your Project Contact. Be sure to indicate the name and code of the BOLD project to which your data are being submitted if this is an ongoing project.

Digital images of specimens and the corresponding Image Submission Form can be submitted directly to BOLD using the online image submission procedure. Refer to the BOLD Image Submission Protocol on the CD accompanying this sampling package. Alternatively, images can be saved on a CD and sent to the BOLD team <mmilton@uoguelph.ca>.

For detailed information on the BOLD data structure and submission procedures, please refer to the BOLD Data Submission Protocol on the CD accompanying this sampling package.