

**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.

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## **DEPARTMENT OF ECOLOGY**

Environmental Assessment Program

January 3, 2012

TO:	Puget Sound Assessment and Monitoring Program Sediment Component E-mail List
THROUGH:	Robert F. Cusimano, Section Manager, Environmental Assessment Program 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) 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.

cc: Sandra Weakland, Environmental Assessment Program Valerie Partridge, Environmental Assessment Program Kathy Welch, Environmental Assessment Program Ed Long, Environmental Assessment Program Stuart Magoon, Environmental Assessment Program Bill Kammin, Environmental Assessment Program Tom Gries, Environmental Assessment Program Joyce Mercuri, Toxics Cleanup Program Joel Baker, University of Washington-Tacoma Eric Stein, Southern California Coastal Water Research Project Peter Miller, Canadian Centre for DNA Barcoding Bonnie Becker, University of Washington-Tacoma

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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 Rejected	Alternate 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.

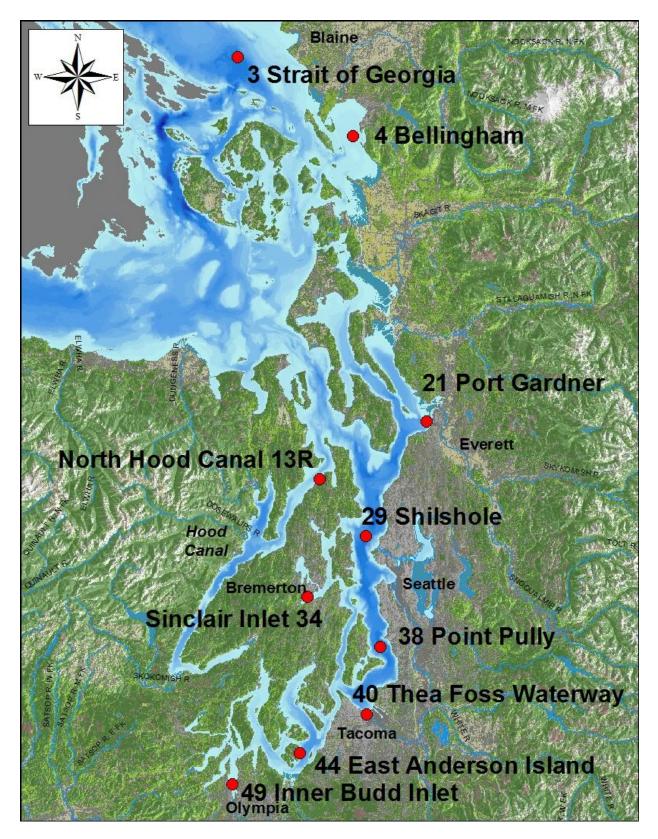


Figure 1. PSAMP 10 long-term temporal sediment monitoring stations in Puget Sound.

Station	Location	Target (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 Grain size – September 2011 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/ Sandra Weakland			
Schedule					
Summary statistics, 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 published every 5 <sup>th</sup> year; next			
Final (all reviews de publications coordin		report after 2015 sampling)			
Final report due on	web				

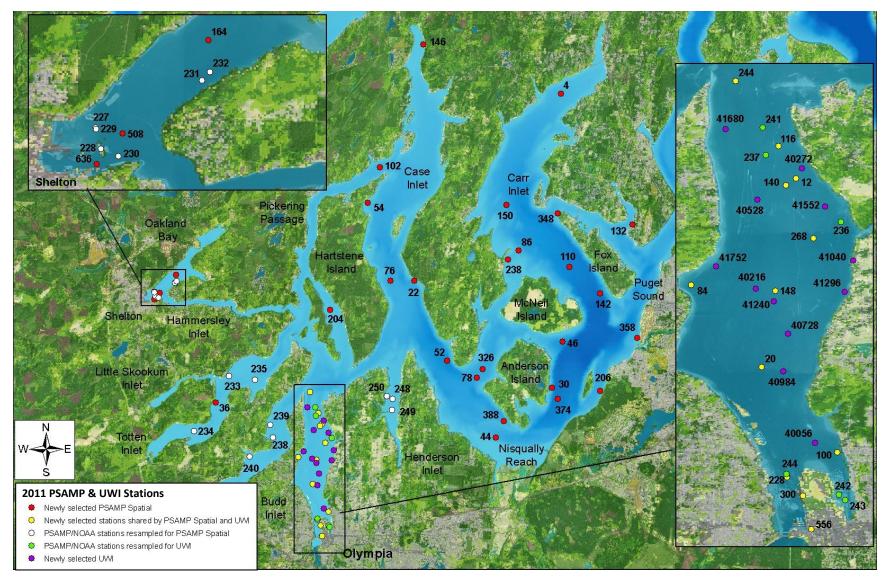


Figure 2. 73 station locations for Ecology's 2011 PSAMP Spatial/Temporal and Urban Waters Initiative Monitoring in the South Puget Sound Sediment Monitoring Region and Budd Inlet.

Originating Project	Station	PSAMP /UWI Label ID	Location	Longitude	Latitude	PSAMP Spatial/ Temporal	UWI	Resampled PSAMP/ NOAA	Dioxin/ Furan	Bar 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 Anderson Island	122.6720	47.1540	Х				
PSAMP Spatial	36	PS00036	Totten Inlet	123.0168	47.1365	Х				
PSAMP Spatial	44	PS00044	Nisqually Reach	122.7283	47.1179	Х				
PSAMP Spatial	46	PS00046	East Anderson Island	122.6625	47.1863	Х				Х
PSAMP Spatial	52	PS00052	Nisqually 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 Passage	122.7497	47.1595	Х				
PSAMP Spatial	86	PS00086	Carr Inlet	122.7104	47.2494	Х				
PSAMP Spatial	102	PS00102	Pickering Passage	122.8554	47.3049	Х				
PSAMP Spatial	110	PS00110	Carr Inlet	122.6572	47.2390	Х				
PSAMP Spatial	132	PS00132	Hale 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 Bay	123.0620	47.2250	Х				
PSAMP Spatial	204	PS00204	Pickering Passage	122.9024	47.2039	Х				
PSAMP Spatial	206	PS00206	East Anderson Island	122.6223	47.1524	Х				Х
PSAMP Spatial	238	PS00238	Drayton Passage	122.7208	47.2428	Х				
PSAMP Spatial	326	PS00326	Drayton Passage	122.7438	47.1657	Х				
PSAMP Spatial	348	PS00348	Hale Passage	122.6711	47.2760	Х				Х
PSAMP Spatial	358	PS00358	East Anderson Island	122.5859	47.1903	Х				
PSAMP Spatial	374	PS00374	East Anderson Island	122.6658	47.1461	Х				
PSAMP Spatial	388	PS00388	Nisqually 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 Project	Station	PSAMP /UWI Label ID	Location	Longitude	Latitude	PSAMP Spatial/ Temporal	UWI	Resampled PSAMP/ NOAA	Dioxin/ Furan	Bar coding
PSAMP Spatial	508	PS00508	Port of Shelton	123.0786	47.2119	Х				Х
PSAMP Spatial	636	PS00636	Port of 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 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 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 Olympia	122.9055	47.0526	Х	Х		Х	
PSAMP Spatial	556	PSUW556	Port of Olympia	122.9036	47.0451	Х	Х		Х	
PSAMP/NOAA	227	PSNO227	Port of Shelton	123.0841	47.2126	Х		Х	Х	Х
PSAMP/NOAA	228	PSNO228	Port of Shelton	123.0828	47.2097	Х		Х		
PSAMP/NOAA	229	PSNO229	Port of Shelton	123.0839	47.2124	Х		Х		
PSAMP/NOAA	230	PSNO230	Oakland Bay	123.0793	47.2088	Х		Х		
PSAMP/NOAA	231	PSNO231	Oakland Bay	123.0630	47.2194	Х		Х		
PSAMP/NOAA	232	PSNO232	Oakland 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 Inlet	122.8355	47.1429	Х		Х		Х
PSAMP/NOAA	249	PSNO249	Henderson Inlet	122.8358	47.1351	Х		Х		
PSAMP/NOAA	250	PSNO250	Henderson Inlet	122.8413	47.1445	Х		Х		
PSAMP/NOAA	266	PSNO266	Hale 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 Project	Station	PSAMP /UWI Label ID	Location	Longitude	Latitude	PSAMP Spatial/ Temporal	UWI	Resampled PSAMP/ NOAA	Dioxin/ Furan	Bar coding
PSAMP/NOAA	242	UWNO242	Port of Olympia	122.8974	47.0529		Х	Х	Х	
PSAMP/NOAA	243	UWNO243	Port of Olympia	122.8959	47.0516		Х	Х		
PSAMP/NOAA	244	UWNO244	Port of 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 Program	BI-S7		Budd Inlet	122.9132	47.0591				Х	
Toxic Cleanup Program	BI-S30		Budd Inlet	122.8946	47.0477				Х	
Toxic Cleanup Program	OB-10		Oakland Bay	123.0496	47.2376				Х	
Toxic Cleanup Program	OB-12.5		Oakland 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 2,4-Dimethylphenol 2-Methylphenol 4-Methylphenol	PBDE-154 PBDE- 183 PBDE- 184 PBDE-191	PCB-52 PCB-66 PCB-77 PCB-101
Phenol Phenol, 4-Nonyl-	PBDE-209 Polychlorinated	PCB-105 PCB-118 PCB-126
<b>Phthalate Esters</b> Bis(2-Ethylhexyl) Phthalate Butylbenzylphthalate	Biphenyls	PCB-128 PCB-138 PCB-153
Diethylphthalate Dimethylphthalate Di-N-Butylphthalate	PCB-1016 PCB-1221 PCB-1232	PCB-169 PCB-170 PCB-180
Di-N-Octyl Phthalate Polybrominated	PCB-1242 PCB-1248 PCB-1254	PCB-187 PCB-195 PCB-206
<b>Diphenylethers</b> PBDE- 47 PBDE– 49	PCB-1260 PCB-1262 PCB-1268	PCB-209 Added in 2000
PBDE- 66 PBDE- 71 PBDE- 99 PBDE-100 PBDE- 138 PBDE-153	Congeners PCB-8 PCB-18 PCB-28 PCB-44	Bisphenol A Tri(2-chloroethyl)phosphate (TCEP) Triclosan 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 Grain size – September 2011 Toxicity – March 2012 Taxonomy – March 2012 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 Initiative: Budd Inlet	AMP Spatial/Temp	oral and Urban Waters			
Author lead		Maggie Dutch (PSAMP)/ 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 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 Sample	Container	Preservation	Maximum Holding Time
Nitrogen containing and organophosphorus pesticides, pyrethroids, and herbicides	8 oz	1 8-oz certified organic- free wide-mouth glass jar with Teflon-lined lid	Store and transport all samples at 0-6°C.	14 days from collection to extraction at 0-6°C, 1 year if frozen at ≤18°C; 40 days from extraction to analysis

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

Parameter	Expected Range of Results	Extraction Method	Clean-Up Method	Analysis Method	Technique/ Instrument	Required Reporting Limit
Nitrogen containing and organophosphorus pesticides, pyrethroids	Unknown			SW-846 Method 8270	Gas Chromatography Mass Spectrometer – GC/MS	50-1000 ug/kg (nitrogen), 10-1000 ug/kg (organophos)
Herbicides	Unknown			SW-846 Method 8270 and 8151 with modifications as per EPA Region 10 Herbicide Procedure for Soils and Drinking and Raw Source Waters	Gas Chromatography Mass Spectrometer – GC/MS or Gas Chromatography Electron 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 Replicate (Split Sample)	Analytical (Laboratory) Replicate	Laboratory Control Sample	Reference Material <sup>1</sup>	Method Blank	Matrix Spike (and Matrix Spike Duplicates)	Surrogate Spike
Measurement Frequency		Duplicate analysis for 5% of samples	Triplicate analysis/batch of 20 samples for grain size and TOC. Duplicate analysis/batch for metals and organics samples.	1/batch of 20	1/batch of 20	1/batch of 20	1/batch of 20	every organics sample, blank, and QC sample (minimum of 3 for neutrals, 3 for acids)
MQO measured		RPD	RSD or RPD	% recovery limits	% recovery limits	comparison of analyte concentration in blank to quantification limit	% recovery limits	% recovery limits
Nitrogen containing and organophosphorus pesticides, pyrethroids, 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 Sample	Container	Preservation	Maximum Holding Time
Pharmaceuticals and Personal Care Products (PPCPs)	8 oz	8 oz HDPE internally certified by contract lab	Wrap in aluminum foil and place in ice chest with dry ice immediately after field collection. Freeze as soon as possible. Store in dark at less than -10°C until analyzed	* Freezing encouraged to minimize degradation. Extract within 48 hours if not frozen or within 7 days of collection if frozen. Extract within 48 hours of removal from freezer. Analyze extracts within 40 days of extraction.
Perfluorinated Chemicals (PFCs)	8 oz	8 oz HDPE internally certified by 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 Range of Results	Extraction Method	Clean-Up Method	Analysis Method	Technique/ Instrument	Required Reporting Limit
Pharmaceuticals and Personal Care Products (PPCPs)	Unknown	Sonication with aqueous buffered acetonitrile and pure acetonitrile, concentrate then dilute with ultra pure water.	Solid-phase extraction cartridge then filtered	USEPA 1694	HPLC/ESI-MS/MS. High performance liquid chromatography with triple quadrupole mass spectrometer in positive and negative electrospray ionization modes using isotope dilution and internal standard quantitation techniques	1-1,000 μg/kg dry weight
Perfluorinated Chemicals (PFCs)	Unknown	Shake extraction with dilute acetic acid solution then methanolic ammonium hydroxide solution. Combine supernatants and treat with ultra pure carbon powder and diluted with ultra pure water.	Weak anion exchange sorbent solid- phase extraction	MLA-041. Internal Axys method	HPLC/ESI-MS/MS. High performance liquid chromatography with triple quadrupole mass spectrometer in negative electrospray ionization mode using internal standard.	0.1 μg/kg 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 Replicate (Split Sample)	Analytical (Laboratory) Replicate	Laboratory Control Sample	Reference Material <sup>1</sup>	Method Blank	Matrix Spike (and Matrix Spike Duplicates)	Surrogate Spike
Measurement Frequency		Duplicate analysis for 5% of samples	Triplicate analysis/batch of 20 samples for grain size and TOC. Duplicate analysis/batch for metals and organics samples.	1/batch of 20	1/batch of 20	1/batch of 20	1/batch of 20	every organics sample, blank, and QC sample (minimum of 3 for neutrals, 3 for acids)
MQO measured	RPD	RPD	RSD or RPD	% recovery limits	% recovery limits	comparison of analyte concentration in blank to quantification limit	% recovery limits	% recovery limits
Pharmaceuticals and Personal Care Products (PPCPs)	RPD ≤ 20%	RPD ≤ 20%	Compound specific RPD $\leq 40\%$	compound specific	NA	Analyte concentration $<$ MDL; if $\ge$ MDL, lowest analyte concn. must be $\ge 10x$ method blank concn.	NA	compound specific
Perfluorinated Chemicals (PFCs)	RPD <u>&lt;</u> 20%	RPD ≤ 20%	Compound specific RPD ≤ 40%	compound specific	NA	Analyte concentration $<$ MDL; if $\ge$ MDL, lowest analyte concn. must be $\ge 10x$ method blank concn.	Recovery compound specific; 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

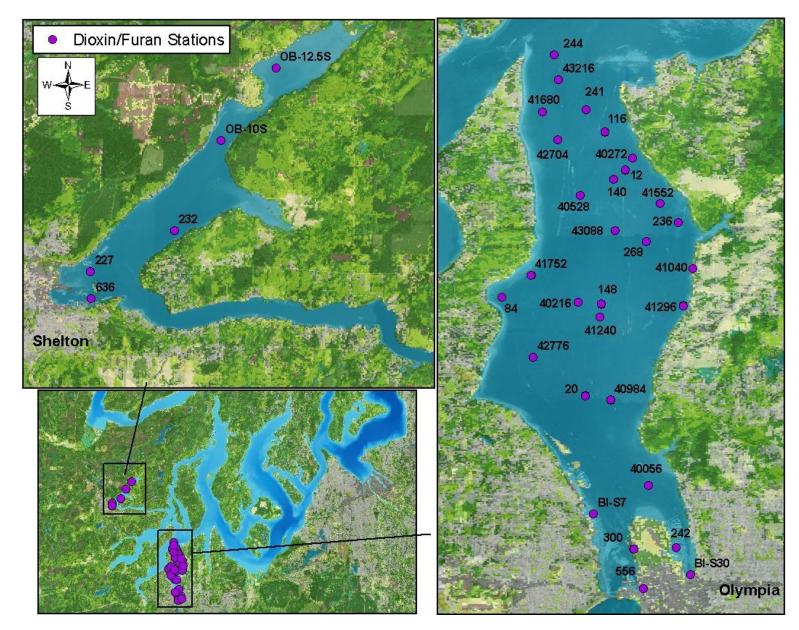


Figure 3. Station locations for Ecology's 2011 sediment sampling in Budd Inlet and Oakland Bay for dioxins and furans.

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

PCDD/F congener	Sediment Target EQL (ng/Kg 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 Calibration (r)	Continuing calibration (% recovery)	EQL	Field	l blanks		oratory ks/batch	&/c spił	duplicates or matrix ces/batch 6 RPD)		LCS or SRM (% recovery)
			MQO	No.	MQO	No.	MQO	No.	MQO	No.	$MQO^3$
Dioxins/furans Individual congeners (ng/kg dry weight)	See Method (EPA, 1994)	See Method (EPA, 1994)	Varies 1.0-5.0			1	<0.5RL	1	< 50	1	Specified by method or within 2 standard deviations of 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 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 +9 (	1 new 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, 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 No.	Rep	Date	NAI	n Target D 1983 I Minutes Longitude	DGPS L Trimble 1 (1-m. ac NAD 1983, De Latitude	NT300D curacy)	GPS Time	Dist. to Target m.	Meter Wheel Depth m.	Predicted Tide (m.): Nearest Station	Predicted Mudline Depth, m. (MLLW)	GPS Status HDOP <2 good	Comments
UW40528, 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, 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, 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, BI-300, 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, 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 No.	Rep	Date	NAI Decima	<u>n Target</u> D 1983 Il Minutes	<u>DGPS L</u> Trimble l (1-m. ac NAD 1983, De	NT300D ccuracy) ccimal Minutes	GPS Time	Dist. to Target m.	Meter Wheel Depth m.	Predicted Tide (m.): Nearest Station	Predicted Mudline Depth, m. (MLLW)	GPS Status HDOP <2 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, 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, 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, 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, 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 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 No.	Rep	Date	NAI	n <u>Target</u> D 1983 I Minutes	<u>DGPS L</u> Trimble l (1-m. ac NAD 1983, De	NT300D curacy)	GPS Time	Dist. to Target m.	Meter Wheel Depth m.	Predicted Tide (m.): Nearest Station	Predicted Mudline Depth, m. (MLLW)	GPS Status HDOP <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, 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, 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, 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, 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 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, 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 No.	Rep	Date	NAI Decima	<u>n Target</u> D 1983 I Minutes	DGPS L Trimble l (1-m. ac NAD 1983, De	NT300D curacy) cimal Minutes	GPS Time	Dist. to Target m.	Meter Wheel Depth m.	Predicted Tide (m.): Nearest Station	Predicted Mudline Depth, m. (MLLW)	GPS Status HDOP <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, 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, 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, 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, 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, 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, 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 No.	Rep	Date	NAI Decima	<u>n Target</u> D 1983 1l Minutes	<u>DGPS L</u> Trimble l (1-m. ac NAD 1983, De	NT300D curacy) cimal Minutes	GPS Time	Dist. to Target m.	Meter Wheel Depth m.	Predicted Tide (m.): Nearest Station	Predicted Mudline Depth, m. (MLLW)	GPS Status HDOP <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, BI-556, 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, 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, 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 No.	Rep	Date	NAI	n Target D 1983 al Minutes Longitude	DGPS L Trimble 1 (1-m. ac NAD 1983, De Latitude	NT300D curacy)	GPS Time	Dist. to Target m.	Meter Wheel Depth m.	Predicted Tide (m.): Nearest Station	Predicted Mudline Depth, m. (MLLW)	GPS Status HDOP <2 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 No.	Rep	Date	NAI	n Target D 1983 al Minutes Longitude	DGPS L Trimble l (1-m. ac NAD 1983, De Latitude	NT300D curacy)	GPS Time	Dist. to Target m.	Meter Wheel Depth m.	Predicted Tide (m.): Nearest Station	Predicted Mudline Depth, m. (MLLW)	GPS Status HDOP <2 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 offshore
	2				47 16.7264	122 51.8854	1014	0.9	13.0	-0.70	-13.7	1.0	commercial 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 No.	Rep	Date	NAI	n Target D 1983 al Minutes Longitude	DGPS L Trimble 1 (1-m. ac NAD 1983, De Latitude	NT300D curacy)	GPS Time	Dist. to Target m.	Meter Wheel Depth m.	Predicted Tide (m.): Nearest Station	Predicted Mudline Depth, m. (MLLW)	GPS Status HDOP <2 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 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, OB-227S, 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, OB-232S, 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 No.	Rep	Date	Station Target       NAD 1983       Decimal Minutes       Latitude     Longitude		DGPS Location Trimble NT300D (1-m. accuracy) NAD 1983, Decimal Minutes Latitude Longitude		GPS Time	Dist. to Target m.	Meter Wheel Depth m.	Predicted Tide (m.): Nearest Station	Predicted Mudline Depth, m. (MLLW)	GPS Status HDOP <2 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, 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, 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, FS00006, OB-636S, 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 No.	Rep	Date	Station Target       NAD 1983       Decimal Minutes       Latitude     Longitude		DGPS Location Trimble NT300D (1-m. accuracy) NAD 1983, Decimal Minutes Latitude Longitude		GPS Time	Dist. to Target m.	Meter Wheel Depth m.	Predicted Tide (m.): Nearest Station	Predicted Mudline Depth, m. (MLLW)	GPS Status HDOP <2 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 No.	Rep	Date	NAI	n Target D 1983 al Minutes Longitude	DGPS L Trimble M (1-m. ac NAD 1983, De Latitude	NT300D curacy)	GPS Time	Dist. to Target m.	Meter Wheel Depth m.	Predicted Tide (m.): Nearest Station	Predicted Mudline Depth, m. (MLLW)	GPS Status HDOP <2 good	Comments
	3				47 07.3310	122 58.6254	1149	1.1	12.9	2.62	-10.3	1.2	
PSUW116 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 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





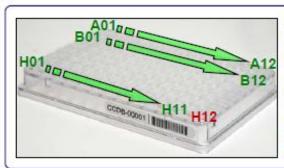
If samples are prone to spontaneous displacement because of static electricity (e.g., dry insect legs), sampling wells should be pre-filled with 30  $\mu$ l (microlitres) of 95% Ethanol, e.g., using a multi channel pipettor. If a pipettor is not available, add one drop of Ethanol to each well using an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

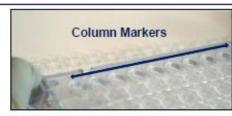


Start the sampling process with A01 (row 1) and proceed in alphanumerical order to A12 (left to right). When done with the first row, proceed to the second row (B01) and repeat the process until all 12 rows are filled. Do not leave empty wells in the middle of the plate. While sampling, remove only one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

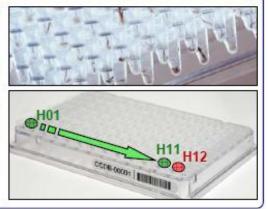
Avoid sampling from body parts containing scales, hairs or bristles, when possible. Avoid sampling from digestive tracts or from areas which may have been in contact with digestive tract contents.

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.

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	>	Canadian Cer	tre for DNA E	Barcoding	y .	Select type of se	moling medium	microplete	
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	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			
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			-	CCDB	number(s) as	shown here:	CCDB-00001-00	007_Record.sl	•
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			-	with th	e correspond	ing BOLD record and col	lection voucher	specimen	
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	CCD8-00001-007 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.

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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.