DEPARTMENT OF
ECOLOGY
State of Washington

2012 Addendum to Quality Assurance Project Plan

The Puget Sound Assessment and Monitoring Program/Urban Waters Initiative: Sediment Monitoring in the San Juan Islands and Port Gardner/ Everett Harbor

December 2011

Publication No. 09-03-121-Addendum3

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/0903121Addendum3.html. Data are available on Ecology's Environmental Information Management (EIM) website at www.ecy.wa.gov/eim/index.htm. Search Study ID UWI2012.

Original Publication

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

Publication No. 09-03-121.

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

Authors and Contact Information

Margaret Dutch, Sandra Weakland, Valerie Partridge, Kathy Welch, Ed Long
Environmental Assessment Program
Washington State Department of Ecology
Olympia, Washington 98504-7710

For more information contact:

Communications Consultant
Environmental Assessment Program
P.O. Box 47600
Olympia, WA 98504-7600
Phone: 360-407-6834

DEPARTMENT OF ECOLOGY

Environmental Assessment Program

December 2011

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: 2012 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-Addendum3

The Washington State Department of Ecology's (Ecology's) Marine Sediment Monitoring Team (MSMT) will conduct sediment sampling in April and June, 2012, 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 will be conducted at 10 PSAMP Long-term/Temporal monitoring stations located throughout Puget Sound. June's PSAMP Spatial/Temporal Monitoring Program sampling will be conducted in the MSMT's San Juan Islands sediment monitoring region. Intensive sampling will also occur in the vicinity of Port Gardner/Everett Harbor, as part of the UWI program. Additional samples will also be collected in June to measure the following:

- Concentrations of Pharmaceuticals and Personal Care Products (PPCPs) and Perfluorinated Chemicals (PFCs) from all PSAMP and UWI stations to be analyzed at the University of Washington-Tacoma (UW-T) (*pending further discussion and arrangements with UW-T staff to analyze these samples*).
- Dioxin and furan concentrations in selected stations from Port Gardner/Everett Harbor sediments as a special project for Ecology's Toxic Cleanup Program *(pending project submittal from TCP).*
- 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 about all sampling locations, parameters, quality assurance, and sampling/analysis schedules for each project that will be conducted in 2012.

As with past sampling events, Ecology makes every effort to coordinate these sediment sampling efforts with sampling that may be planned by regional stakeholders. All inquiries about this sediment monitoring work and potential partnership sampling with Ecology can be directed to me at margaret.dutch@ecy.wa.gov or 360-407-6021.

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

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Ongoing Monitoring Programs

April 2012 – 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.

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

June 2012 – Spatial Sediment Monitoring in the San Juan Islands

Sampling in June 2012 will be conducted for two on-going sediment monitoring efforts, including Ecology's PSAMP Spatial/Temporal Monitoring Program and UWI. A total of 70 stations will be sampled for these two projects with sampling occurring in the San Juan Island region and Port Gardner/Everett Harbor urban bay sampling frame, respectively.
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 to 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: 40 randomly selected locations in the San Juan Island Sediment Monitoring Region (Figure 2, Table 4). Alternate station locations are available in case a station location cannot be sampled (Figure 3, Table 5).

Parameters Sampled: Field measurements, toxicity, macroinvertebrate abundance, grain size, total organic carbon, metals, and organic chemical contaminants (Table 6).

Project Schedule: Outlined in Table 7.

Link to further information about this regional monitoring program: www.ecy.wa.gov/programs/eap/psamp/SpatialMon/Spatial.htm.

Ecology's Urban Waters Initiative (UWI) Monitoring – Port Gardner/Everett Harbor

Purpose: To characterize sediment quality in the UWI Port Gardner/Everett Harbor sampling frame and to set a baseline that can be used to determine change over time in the future.

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 will be intensively sampled in Port Gardner/Everett Harbor (Figure 4, Table 8). Alternate station locations are proposed in case a station location cannot be sampled (Figure 4, Table 9).

Parameters Sampled: Field measurements, toxicity, macroinvertebrate abundance, grain size, total organic carbon, metals, and organic chemical contaminants (Table 6).

Project Schedule: Outlined in Table 7.

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

Special Projects – June 2012

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

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 Port Gardner/Everett Harbor sampling frame. Extra sediment will be collected from each June 2012 sampling location and turned over to partners at the University of Washington-Tacoma (UW-T) Environmental Science department as a continuing 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 Washington State accreditation for conduct of these analyses (http://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^2 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: 70 stations, as per PSAMP Spatial/Temporal and UWI Monitoring Programs (Figures 2,4; Tables 4,8).

Parameters Sampled: Field measurements, 119 PPCPs, 13 PFCs (Table 10). 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 11.

Laboratory Analysis and Reporting Requirements: Outlined in Table 12.

Field and Laboratory Measurement Quality Objectives: Outlined in Table 13.

Project Schedule: Samples will be collected and delivered to UW-T personnel at completion of sampling in June 2012. Dr. Joel Baker (jebaker@u.washington.edu) and Dr. Joyce Dinglasan-Panlilio (jdingpan@u.washington.edu) can be contacted for details of the project schedule.

Chlorinated Dioxins and Furans in Port Gardner/Everett Harbor (Project Pending)

Purpose:

- To measure concentrations of 17 chlorinated dioxin and furan congeners in surface sediments of Port Gardner/Everett Harbor.
- To use results to estimate concentrations that might represent background conditions for Port Gardner/Everett Harbor.

Partnership: Ecology's Toxic Cleanup Program project lead: to be determined; Ecology's Environmental Assessment Program project lead: to be determined.

Sampling Details: To be determined.

Station Locations: To be determined.

Parameters Sampled: Field measurements, grain size, total organic carbon, 17 PCDD/F congeners (Table 14).

Sample Volumes and Preservation for Laboratory Analysis: Outlined in Table 15.

Laboratory Analysis and Reporting Requirements: Outlined in Tables 16 and 17.

Field and Laboratory Measurement Quality Objectives: Outlined in Table 18.

Project Schedule: Outlined in Table 19.

Link to further information about toxic cleanup work in Port Gardner/Everett Harbor: http://www.ecy.wa.gov/programs/tcp/sites_brochure/psi/everett/psi_everett.html

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) (http://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 (http://www.boldsystems.org/views/login.php).

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 A).

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 the Puget Sound area.

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 with 5% glycerin, sorted and identified to the species level, and their tissue harvested and shipped to the Centre as per developed protocols (Appendix B). Data will then be incorporated into the BOLD database and publically available.

Station Locations: Benthic invertebrates will be collected from PSAMP Long Term/Temporal, PSAMP Spatial/Temporal, and UWI Monitoring Program stations. Station locations are identified in Figures 1,2,4; Tables 1,4,8.

Parameters Sampled: Marine benthic invertebrates.

Sample Volumes and Preservation for Laboratory Analysis: Invertebrates will be 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 B).

Laboratory Analysis and Reporting Requirements: All laboratory analysis and reporting requirements for collection, sieving, and sorting of benthic invertebrate samples will follow those in the established PSAMP/UWI project plan (Dutch et al., 2009) and those outlined in International Barcode of Life Project data submission package (Appendix B).

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, and the schedule will be adjusted as needed based on availability of volunteers. Collection and sieving of invertebrates in the field, and sorting of barcode samples in the lab, will be conducted by student interns from Dr. Bonnie Becker's lab at the University of Washington-Tacoma, as well as citizen volunteers, supervised by MSMT staff. 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 A, has yet to be determined.

Future Sediment Monitoring

Future monitoring locations and sampling dates for the PSAMP and UWI programs listed above are indicated in the schedule in Table 20.

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

Figures and Tables

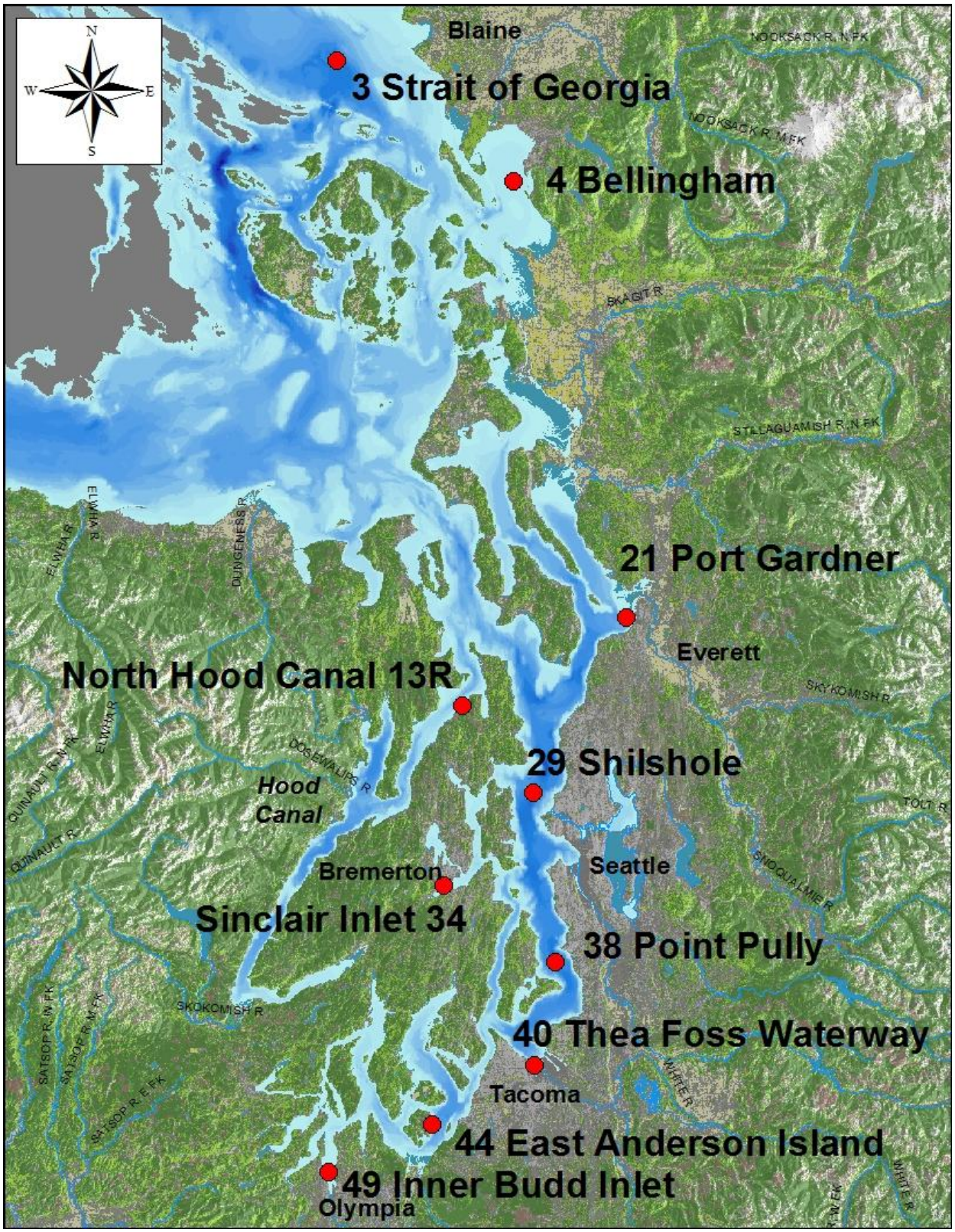


Figure 1. PSAMP 10 Long-term/Temporal sediment monitoring stations in Puget Sound.

Table 1. Location (latitude/longitude) for the 2012 PSAMP Sediment Component Long-term/Temporal Monitoring Element.

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

Table 2. Parameters measured in Puget Sound sediments for the 2012 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 2012 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 2012
Laboratory analyses completed		Total Organic Carbon – July 2012 Grain size – September 2012 Taxonomy – March 2013
Environmental Information System (EIM) system		
Product	Due date	Lead Staff
EIM data loaded	April 2013	Sandra Weakland
EIM QA	May 2013	Maggie Dutch
EIM complete	June 2013	Sandra Weakland
Final report: 2015 PSAMP Long-Term/Temporal Monitoring		
Author lead		Maggie Dutch
Schedule		
Summary statistics, graphics, and text generated and posted to web		June 2013
Draft due to supervisor		Not applicable: PSAMP long-term/temporal report published every 5th year; next report after 2015 sampling
Draft due to client/peer reviewer		
Draft due to external reviewer		
Final (all reviews done) due to publications coordinator		
Final report due on web		

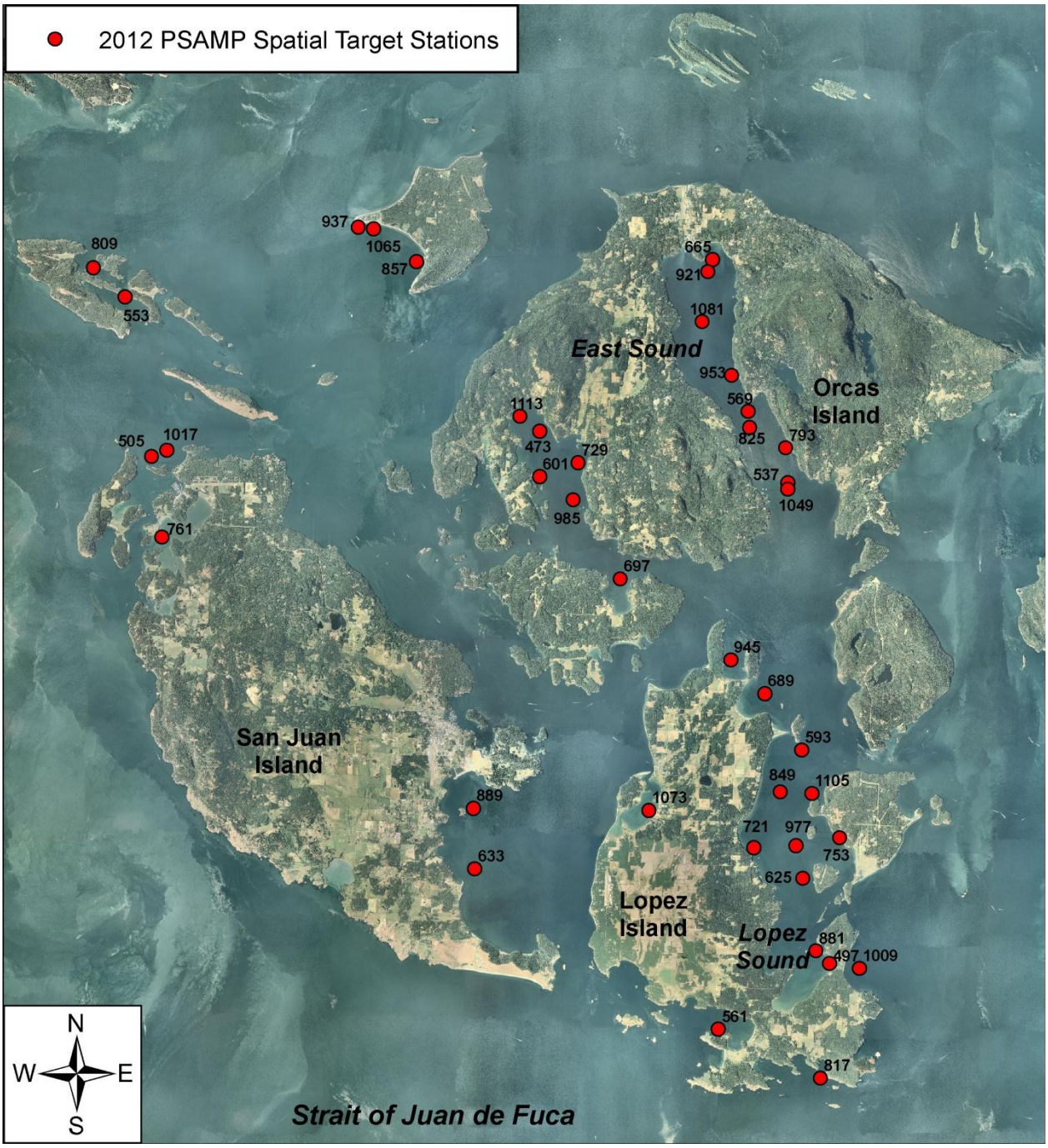


Figure 2. Ecology's 2012 PSAMP Spatial/Temporal Monitoring – Target locations - 40 sediment monitoring stations in the San Juan Island Sediment Monitoring Region.

Table 4. Target location (latitude/longitude) for Ecology's 2012 PSAMP Spatial/Temporal Monitoring Program - 40 stations in San Juan Island sediment monitoring region.

Station	Strata	Location	Target (NAC83, decimal)	
			Latitude	Longitude
473	Rural	West Sound and Massacre Bay	48.63240	-122.97736
497	Rural	Lopez Sound, Hunter and Mud Bay	48.46181	-122.82710
505	Rural	Roche Harbor	48.62000	-123.16795
537	Rural	East Sound	48.61799	-122.85456
553	Rural	Reid Harbor	48.67167	-123.18365
561	Rural	Mackaye Harbor and Outer Bay	48.43927	-122.88072
569	Rural	East Sound	48.64081	-122.87529
593	Rural	Lopez Sound, Hunter and Mud Bay	48.53108	-122.84398
601	Rural	West Sound and Massacre Bay	48.61750	-122.97677
625	Rural	Lopez Sound, Hunter and Mud Bay	48.48925	-122.84137
633	Rural	Griffin and North Bay	48.48910	-123.00259
665	Rural	East Sound	48.68999	-122.89497
689	Rural	Swifts Bay	48.54913	-122.86291
697	Rural	Blind Bay	48.58507	-122.93545
721	Rural	Lopez Sound, Hunter and Mud Bay	48.49876	-122.86590
729	Rural	West Sound and Massacre Bay	48.62250	-122.95796
753	Rural	Lopez Sound, Hunter and Mud Bay	48.50283	-122.82401
761	Rural	Westcott Bay	48.59374	-123.16140
793	Rural	East Sound	48.62938	-122.85616
809	Rural	Prevost Harbor	48.68085	-123.19957
817	Rural	Aleck, Hughes and McArdle Bay	48.42434	-122.82983
825	Rural	East Sound	48.63574	-122.87428
849	Rural	Lopez Sound, Hunter and Mud Bay	48.51719	-122.85383
857	Rural	West of Wadron Island, North and Cowlitz Bay	48.68630	-123.04065
881	Rural	Lopez Sound, Hunter and Mud Bay	48.46575	-122.83404
889	Rural	Griffin and North Bay	48.50882	-123.00422
921	Rural	East Sound	48.68603	-122.89704
937	Rural	West of Wadron Island, North and Cowlitz Bay	48.69692	-123.06996
945	Rural	Shoal Bay	48.55975	-122.88008
953	Rural	East Sound	48.65251	-122.88378
977	Rural	Lopez Sound, Hunter and Mud Bay	48.49992	-122.84539
985	Rural	West Sound and Massacre Bay	48.61031	-122.95993
1009	Rural	Shoal Bight	48.46047	-122.81239
1017	Rural	Roche Harbor	48.62212	-123.16051
1049	Rural	East Sound	48.61584	-122.85452
1065	Rural	West of Wadron Island, North and Cowlitz Bay	48.69651	-123.06243
1073	Rural	Fisherman Bay	48.50996	-122.91799
1081	Rural	East Sound	48.66965	-122.89913
1105	Rural	Lopez Sound, Hunter and Mud Bay	48.51703	-122.83831
1113	Rural	West Sound and Massacre Bay	48.63695	-122.98726

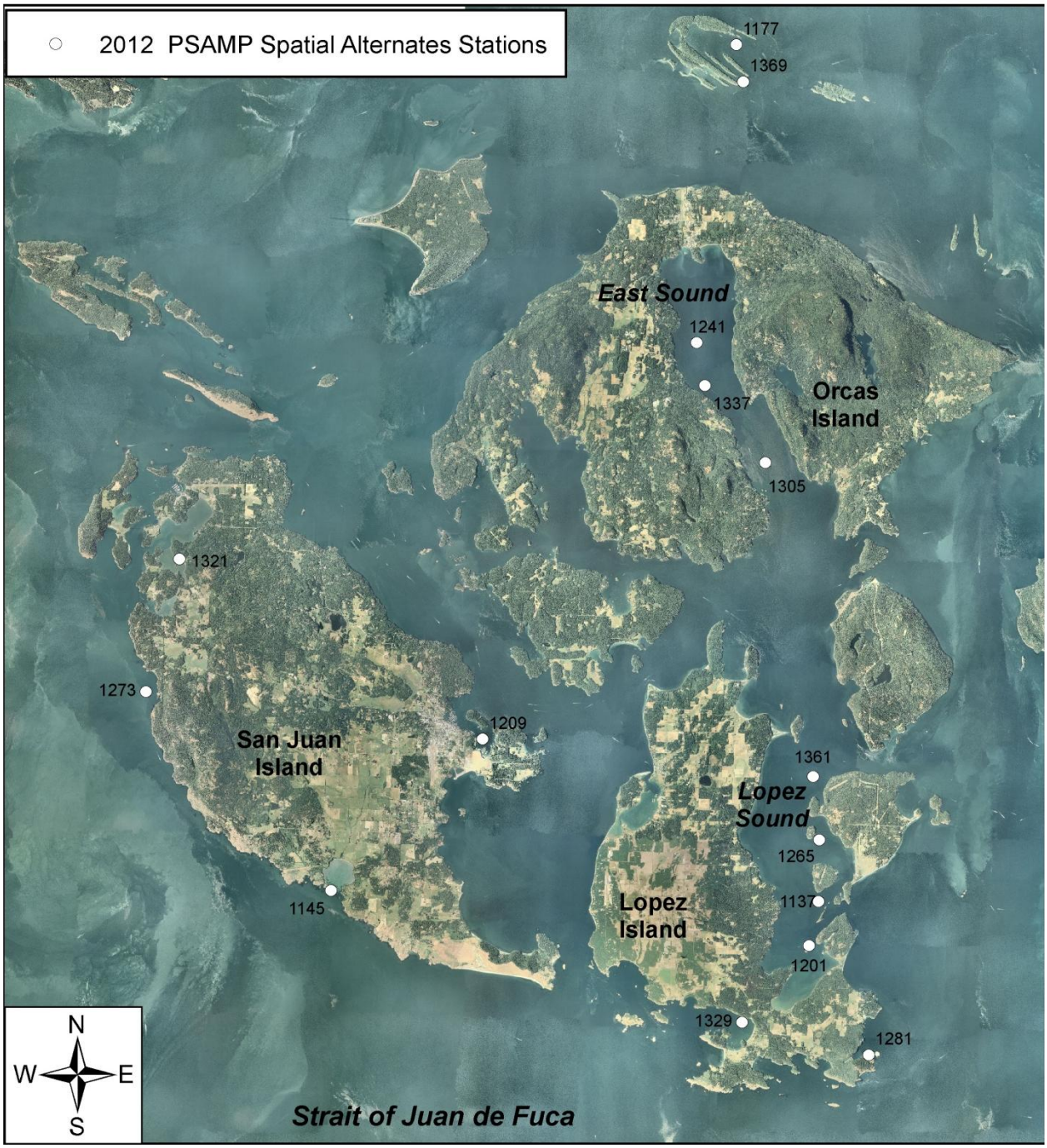


Figure 3. Ecology's 2012 PSAMP Sediment Component Spatial/Temporal Monitoring Element – Alternate locations - 15 sediment monitoring stations in the San Juan Island Sediment Monitoring Region.

Table 5. Alternate locations (latitude/longitude) for Ecology's 2012 PSAMP Sediment Component Spatial/Temporal Monitoring Element - 15 stations in the San Juan Island sediment monitoring region.

Station	Strata	Location	Target (Nac83, Decimal)	
			Latitude	Longitude
1137	Rural	Lopez Sound, Hunter and Mud Bay	48.48188	-122.83306
1145	Rural	Kanaka Bay	48.48050	-123.07279
1177	Rural	Echo and Fossil Bay	48.76118	-122.88609
1201	Rural	Lopez Sound, Hunter and Mud Bay	48.46730	-122.8371
1209	Rural	Friday Harbor	48.53163	-123.00053
1241	Rural	East Sound	48.66333	-122.90117
1265	Rural	Lopez Sound, Hunter and Mud Bay	48.50204	-122.83364
1273	Rural	Andrews Bay	48.54350	-123.16707
1281	Rural	Watmough Bay	48.43220	-122.80612
1305	Rural	East Sound	48.62478	-122.86566
1321	Rural	Westcott Bay	48.58724	-123.15267
1329	Rural	Mackaye Harbor and Outer Bay	48.44164	-122.86891
1337	Rural	East Sound	48.64948	-122.89662
1361	Rural	Lopez Sound, Hunter and Mud Bay	48.52270	-122.83757
1369	Rural	Echo and Fossil Bay	48.74917	-122.88219

Table 6. Parameters measured in Puget Sound sediments for the 2012 PSAMP Sediment Component Spatial/Temporal Monitoring Element and Urban Waters Initiative (UWI).

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
Phenol
Phenol, 4-Nonyl-

Phthalate Esters
Bis(2-Ethylhexyl) Phthalate
Butylbenzylphthalate
Diethylphthalate
Dimethylphthalate
Di-N-Butylphthalate
Di-N-Octyl Phthalate

Polybrominated Diphenylethers
PBDE-47
PBDE-49
PBDE-66
PBDE-71
PBDE-99
PBDE-100
PBDE-138
PBDE-153
PBDE-154
PBDE-183
PBDE-184
PBDE-191
PBDE-209

Polychlorinated Biphenyls

Aroclors
PCB-1016
PCB-1221
PCB-1232
PCB-1242
PCB-1248
PCB-1254
PCB-1260
PCB-1262
PCB-1268

Congeners
PCB-8
PCB-18
PCB-28
PCB-44
PCB-52
PCB-66
PCB-77
PCB-101
PCB-105
PCB-118
PCB-126
PCB-128
PCB-138
PCB-153
PCB-169
PCB-170
PCB-180
PCB-187
PCB-195
PCB-206
PCB-209

Added in 2009
Bisphenol A
Tri(2-chloroethyl)phosphate (TCEP)
Triclosan
Triethyl citrate

Table 7. Proposed schedule for completing the 2012 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 2012
Laboratory analyses completed		Total Organic Carbon – July 2012 Grain size – September 2012 Toxicity – March 2013 Taxonomy – March 2013 Chemistry – March 2013
Environmental Information System (EIM) system		
Product	Due date	Lead Staff
EIM data loaded	April 2013	Sandra Weakland
EIM QA	May 2012	Maggie Dutch
EIM complete	June 2013	Sandra Weakland
Final report: 2012 PSAMP Spatial/Temporal and Urban Waters Initiative: Port Gardner/Everett Harbor		
Author lead		Maggie Dutch (PSAMP)/ Valerie Partridge (UWI)
Schedule		
Summary statistics, graphics, and text generated and posted to web		June 2013
Draft due to supervisor		September 2013
Draft due to client/peer reviewer		October 2013
Draft due to external reviewer		November 2013
Final (all reviews done) due to publications coordinator		December 2013
Final report due on web		January 2014

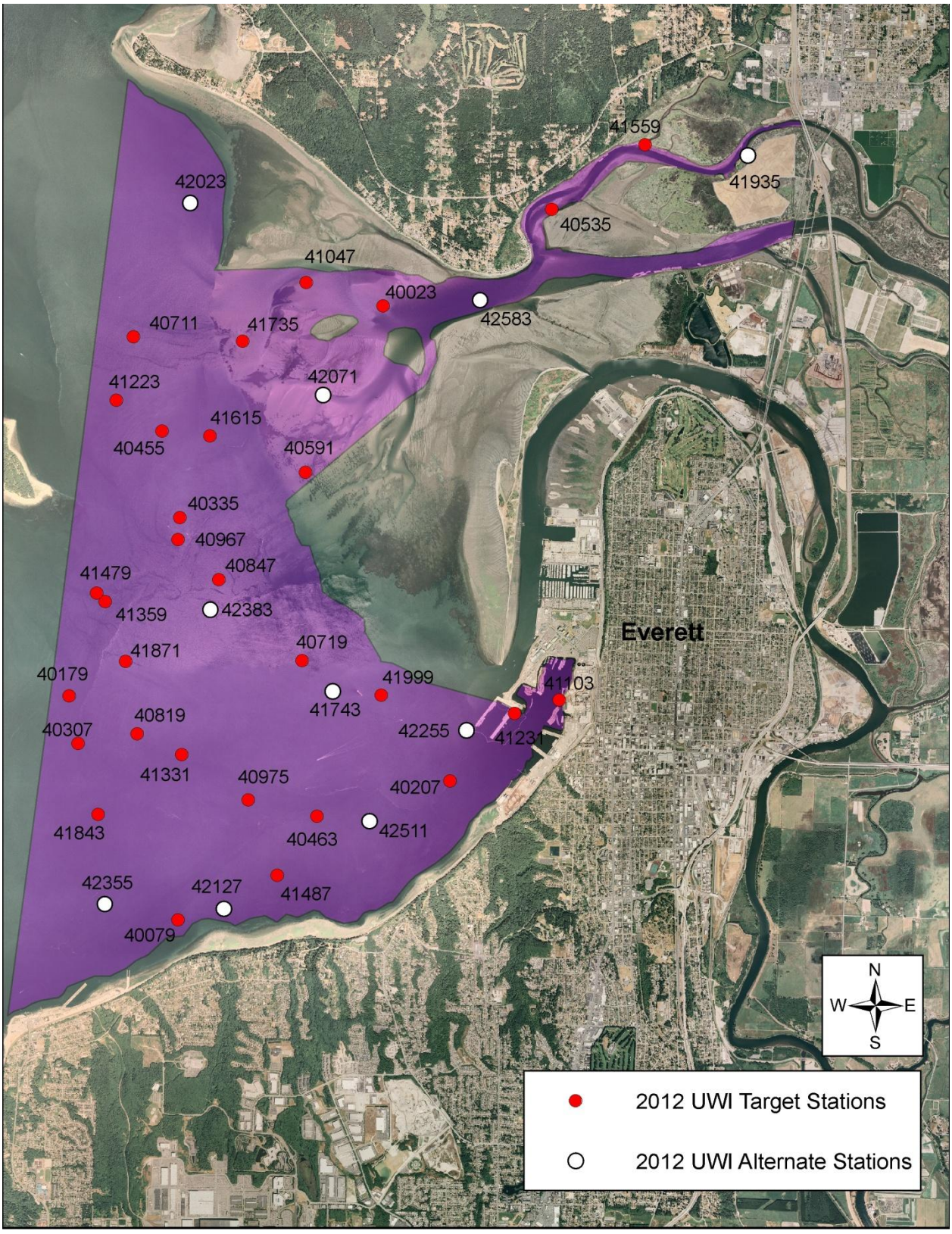


Figure 4. Ecology's 2012 PSAMP Urban Waters Initiative Monitoring - 30 sediment monitoring target stations and 10 alternate stations in Port Gardner/Everett Harbor.

Table 8. Location (latitude/longitude) for Ecology's 2012 Urban Waters Initiative Monitoring Program - 30 target stations in Port Gardner/Everett Harbor.

Station	Strata	Target (Nac83, Decimal)	
		Latitude	Longitude
40023	Rural	48.02659	-122.24986
40079	Rural	47.95991	-122.28059
40179	Rural	47.98380	-122.29893
40207	Rural	47.97551	-122.23749
40307	Rural	47.97868	-122.29727
40335	Rural	48.00329	-122.28179
40455	Rural	48.01256	-122.28495
40463	Rural	47.97142	-122.25867
40535	Rural	48.03742	-122.22310
40591	Rural	48.00846	-122.26178
40711	Rural	48.02266	-122.28990
40719	Rural	47.98817	-122.26163
40819	Rural	47.97988	-122.28787
40847	Rural	47.99670	-122.27528
40967	Rural	48.00091	-122.28202
40975	Rural	47.97300	-122.26977
41047	Rural	48.02892	-122.26236
41103	Harbor	47.98450	-122.22021
41223	Rural	48.01578	-122.29239
41231	Harbor	47.98299	-122.22734
41331	Rural	47.97773	-122.28064
41359	Rural	47.99404	-122.29348
41479	Rural	47.99496	-122.29489
41487	Rural	47.96496	-122.26487
41559	Rural	48.04457	-122.20839
41615	Rural	48.01214	-122.27724
41735	Rural	48.02243	-122.27231
41743	Rural	47.98462	-122.24882
41843	Rural	47.97108	-122.29379
41871	Rural	47.98766	-122.28994

Table 9. Alternate location (latitude/longitude) for Ecology's 2012 Urban Waters Initiative Monitoring Program - 10 stations in Port Gardner/Everett Harbor.

Station	Strata	Target (NAC83, decimal)	
		Latitude	Longitude
42639	Rural	48.01573	-122.26804
42739	Rural	47.99022	-122.29779
42759	Rural	48.02397	-122.27629
42867	Rural	47.96486	-122.29770
42895	Rural	47.98559	-122.26545
43015	Rural	48.01275	-122.29667
43023	Rural	47.97801	-122.24897
43047	Rural	48.04139	-122.27745
43095	Rural	48.02287	-122.25942
43151	Rural	48.00260	-122.26734

Table 10. 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.

Field Measurements

Sediment temperature
Salinity of overlying water

Pharmaceuticals and Personal Care Products

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-Epianhydrotetracycline
4-Epichlortetracycline
4-Epioxytetracycline
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 11. 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
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.
PFCs	8 oz	8 oz HDPE internally certified by contract lab	Refrigerate at 4ºC±2ºC (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 12. 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
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
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 13. Field and laboratory measurement quality objectives (MQOs) 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[1]	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 $\leq$ 20%	RPD $\leq$ 20%	Compound specific RPD $\leq$ 40%	compound specific	NA	Analyte concentration <MDL; if $\geq$ MDL, lowest analyte concn. must be $\geq$ 10x method blank concn.	NA	compound specific
Perfluorinated Chemicals (PFCs)	RPD $\leq$ 20%	RPD $\leq$ 20%	Compound specific RPD $\leq$ 40%	compound specific	NA	Analyte concentration <MDL; if $\geq$ MDL, lowest analyte concn. must be $\geq$ 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 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

Table 14. Parameters measured in Port Gardner/Everett Harbor 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-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
1,2,3,4,7,8,9-HpCDF
1,2,3,4,6,7,8,9-OCDF

Table 15. Sample volumes and preservation for laboratory analysis for dioxin and furan samples.

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. 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 17

Table 17. Target estimated quantitation limits (EQLs) for sediment samples collected from Budd Inlet and Oakland Bay.

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. Measurement quality objectives (MQOs) for field and laboratory quality control samples (per batch ≤ 20 samples).

Parameter	Initial Calibration (r)	Continuing calibration (% recovery)	EQL	Field blanks		Laboratory blanks/batch		Lab duplicates &/or matrix spikes/batch (% 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. Proposed schedule for Ecology's 2012 study of PCDD/Fs in surface sediments of Port Gardner/Everett Harbor.

Field and laboratory work		
Field work completed		June 2012
Laboratory analyses completed		TOC – July 2012 Grain size – September 2012 Chemistry – September 2012
Environmental Information System (EIM) system		
Product	Due date	Lead Staff
EIM data loaded	March 2013	To be determined
EIM QA	April 2013	To be determined
EIM complete	May 2013	To be determined
Final report: 2012 Urban Waters Initiative: Port Gardner/Everett Harbor		
Author lead		To be determined
Schedule		
Draft due to supervisor		November 2012
Draft due to client/peer reviewer		December 2012
Draft due to external reviewer		January 2013
Final (all reviews done) due to publications coordinator		March 2013
Final report due on web		April 2013

Table 20. PSAMP spatial/temporal, PSAMP Long-term/Temporal, Focus, and Urban Waters Initiative sediment sampling schedule (1997-2024).

	Number of Samples Collected															Number of Samples Expected												
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						90 (81 new +9 old)										40										40		
Eastern Strait of Juan de Fuca																	40										40	
Admiralty Inlet																		40										40
Strait of Georgia and Bellingham	100									40										40								
Whidbey Basin											40										40							
Central Sound (north)		100										30										40						
Central Sound (south)													50										40					
South Sound			100												43										40			
Hood Canal								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[1]**										**30[2]**				

* 30 = Grain Size/Total Organic Carbon/Benthos collected; 30+ = Grain Size/Total Organic Carbon/Benthos/Chemistry collected

Focus Studies:

40[1] = 2010 - Pharmaceuticals and Personal Care Products (PPCPs), Perfluorinated Chemicals (PCs) at 10 Long-term/Temporal stations and at 30 UWI Bellingham Bay stations.

30[2] = 2020 - Focus study to be determined.

Appendices

Appendix A. 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 with 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 B. 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 a 5% volume of glycerin to each container to preserve suppleness of the specimens.
- Sample holding times in ETOH:
    - Room temperature – 2-3 months
    - Refrigerated (4°C) – 1 year
    - Freeze (-20°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 (http://www.boldsystems.org) 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 (pemiller@uguelph.ca)or Dirk Steinke (dsteinke@uoguelph.ca) to make these arrangements.
- When barcoding is complete, specimens may be transferred to formalin for fixation and long-term archives.

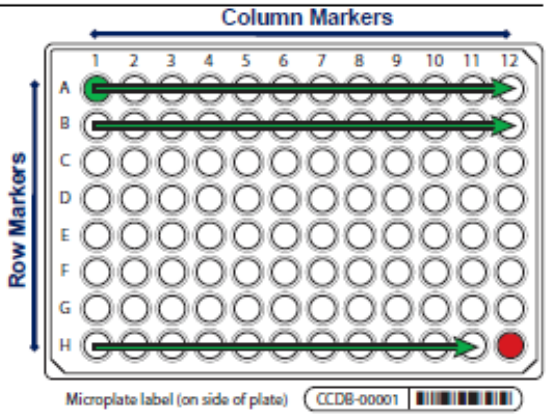
Equipment list – to preserve 10 samples:
20 - ½ gallon or 1 gallon jars for samples
10 gallons 95% ETOH
Coolers/buckets to store samples in

INTERNATIONAL BARCODE OF LIFE PROJECT
MICROPLATE AND DATA SUBMISSION PACKAGE

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).

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.

1. 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.
2. 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 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.
3. The file **ImageData.xls** 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.

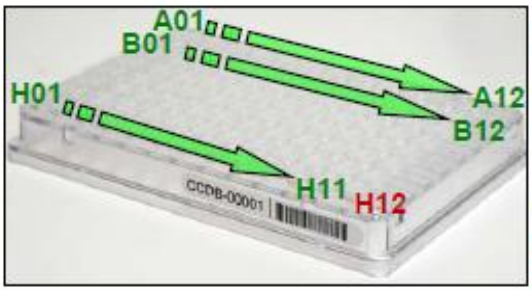
or

If samples are prone to spontaneous **displacement because of static electricity** (e.g., dry insect legs), sampling wells should be pre-filled with **30 µl** (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.

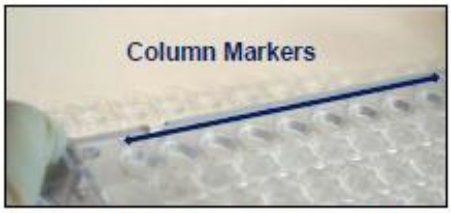


Start the sampling process with **A01** (row 1) and proceed in alphanumerical order to **A12** (left to right). When done with the first row, proceed to the second row (**B01**) and repeat the process until all 12 rows are filled. **Do not leave empty wells in the middle of the plate**. While sampling,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

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



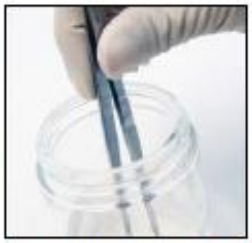
Before beginning the sampling procedure, remove the cap strip from the first row. If required, make sure that wells are pre-filled with fixative (see previous page).

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^3 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.

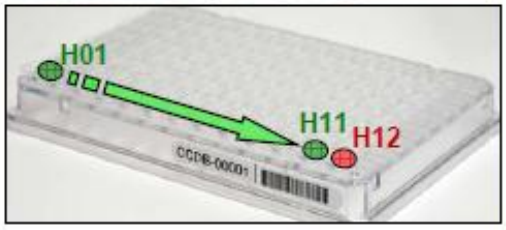
Avoid sampling from body parts containing scales, hairs or bristles, when possible. **Avoid** sampling from digestive tracts or from areas which may have been in contact with digestive tract contents.

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.

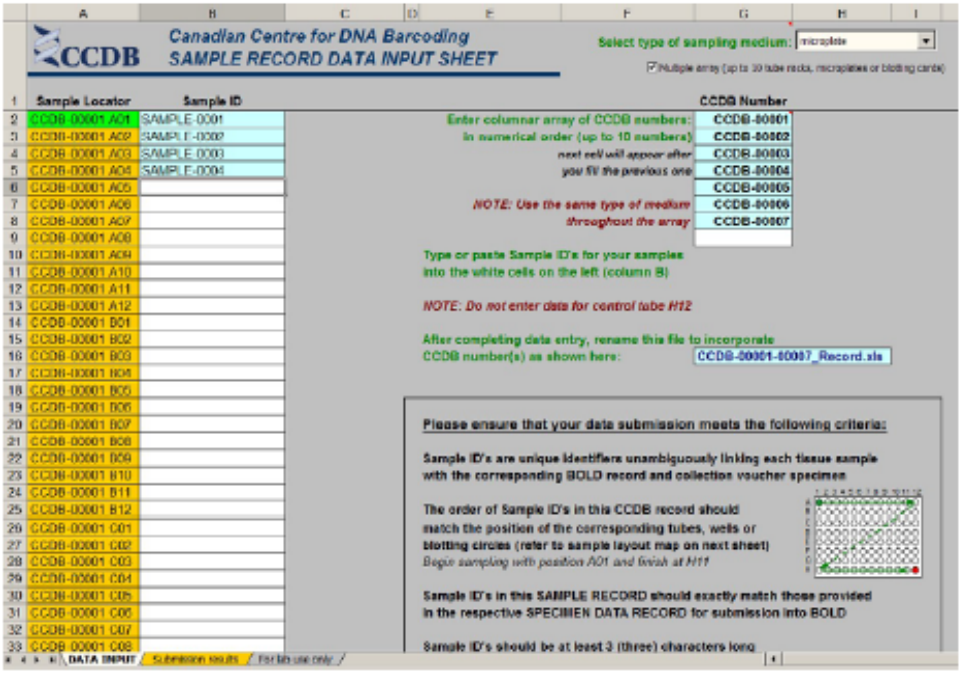
When sampling into the last row (Row H), remember to leave the last well (**H12**) empty. It is OK to add fixative to this well if dispensing with a multi-channel pipettor.

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"**.
2. If intending to fill a multiple plate array, mark the checkbox **"Multiple array..."** in the top right of the sheet.
3. 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.



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.

SUBMITTING TISSUE SAMPLES AND DATA

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 (www.dnabarcoding.ca) or obtained, upon request, from your BOLD Project Manager or from the BIO curator of zoological collections <aborisen@uoguelph.ca>. 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.