

Lower Duwamish Waterway Group

Port of Seattle / City of Seattle / King County / The Boeing Company

TECHNICAL MEMORANDUM: GASTROPOD PILOT SURVEY OF THE LOWER DUWAMISH WATERWAY FINAL

For submittal to

The US Environmental Protection Agency
Region 10
Seattle, WA

The Washington State Department of Ecology
Northwest Regional Office
Bellevue, WA

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**Title and Approval Page
LDW Gastropod Pilot Survey
Quality Assurance Project Plan**

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Acronyms

ACRONYM	Definition
COC	chain of custody
Columbia	Columbia Analytical Services, Inc.
COPC	chemical of potential concern
CPUE	catch per unit effort
dw	dry weight
Ecology	Washington Department of Ecology
EPA	US Environmental Protection Agency
ERA	ecological risk assessment
FC	field coordinator
GPS	global positioning system
LDW	Lower Duwamish Waterway
LDWG	Lower Duwamish Waterway Group
MHHW	mean higher high water
MLLW	mean lower low water
PM	project manager
PSEP	Puget Sound Estuary Program
QA/QC	quality assurance/quality control
QAPP	Quality Assurance Project Plan
RI	Remedial Investigation
ROC	receptor of concern
TM	task manager
TBT	tributyltin
Windward	Windward Environmental LLC

1.0 Introduction

This technical memorandum describes the objectives, methods, and procedures for conducting a pilot survey of gastropods in the Lower Duwamish Waterway (LDW). Data from this effort will be used to support the Phase 2 ecological risk assessment (ERA), as described in the work plan for the Phase 2 Remedial Investigation (RI; Windward 2004b). Section 3.1.5 of the work plan identified the need for additional data to assess risks from tributyltin (TBT) to benthic invertebrates. The pilot survey described in this memo will provide the information needed to finalize the study design to assess risks from TBT through the collection of co-located tissue and sediment samples for TBT analyses.

TBT was identified in the Phase 1 ERA as a chemical of potential concern (COPC) for benthic invertebrates, and will be evaluated using a tissue-based approach¹ in the Phase 2 ERA. Several questions remain unresolved from the Phase 2 work plan regarding which benthic invertebrate tissue should be collected for TBT analyses for comparison to toxicity reference values, which toxicity reference values are most appropriate based on the tissue type, and how these tissues and co-located sediment should be collected. The pilot survey described in this memorandum is designed to provide additional site-specific information to address two of these questions. Following the pilot survey, a data report will be prepared, and then representatives from the US Environmental Protection Agency (EPA), the Washington Department of Ecology (Ecology), and the Lower Duwamish Waterway Group (LDWG) will meet to discuss the pilot survey findings and answer the following questions:

- ◆ Which tissue type should be collected for TBT analysis (i.e., gastropods, a surrogate taxon, or market basket benthic invertebrate tissue samples²)?
- ◆ Where should samples be collected, and how many should be collected, to cover the range of TBT concentrations in sediment, and to gather a sufficient mass of tissue for TBT analysis?
- ◆ Which methods are most appropriate to collect the co-located tissue and sediment as part of the benthic invertebrate fieldwork (see Sections 3.1.3 and 3.2.5 of the Benthic Invertebrate Quality Assurance Project Plan [QAPP] [Windward 2004a])?

Decisions reached at the meeting will be documented in a final study design presented in the final benthic invertebrate QAPP (Windward 2004a). This memorandum, and the technical memorandum presenting the results of the pilot survey, will be attached to that QAPP.

¹ In a tissue-based approach, chemical concentrations are measured in the receptor's tissue and then compared to concentrations associated with adverse effects.

² In the market basket approach, all benthic invertebrates (except larger bivalves and crustaceans) are collected within a targeted sampling area and combined into a single composite tissue sample (see Section 3.1.2 in the benthic invertebrate QAPP (Windward 2004a)).

Elements from EPA guidance for QAPPs (EPA 2002) are included in this memorandum, but a formal QAPP is not warranted because of the qualitative nature of the information to be collected.

The memorandum is organized into the following sections:

- ◆ Section 2 – project organization
- ◆ Section 3 – problem definition/background
- ◆ Section 4 – project/task description and schedule
- ◆ Section 5 - data generation and acquisition
- ◆ Section 6 – sample handling and custody requirements
- ◆ Section 7 - taxonomic identification methods
- ◆ Section 8 – data reporting
- ◆ Section 9 – references
- ◆ Section 10 – oversize figures

2.0 Project Organization

This section presents the overall project organization for the pilot survey, as well as responsibilities of project team members. LDWG, EPA, and Ecology will be involved in all aspects of this project, including discussion, review, and approval of the QAPP, and interpretation of the results of the investigation. EPA and Ecology will be represented by their Project Managers (PMs) for this project, Allison Hiltner and Rick Huey, respectively.

Kathy Godtfredsen will serve as the Windward PM. The Windward PM is responsible for overall project coordination and provides oversight on planning and coordination, work plans, all project deliverables, and performance of the administrative tasks needed to ensure timely and successful completion of the project. She will also be responsible for coordinating with LDWG, EPA, and Ecology on schedule, deliverables, and other administrative details. Dr. Godtfredsen can be reached as follows:

Kathy Godtfredsen
Windward Environmental LLC
200 W. Mercer St., Suite 401
Seattle, WA 98119
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Facsimile: 206.217.0089
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Helle Andersen will serve as the Windward Task Manager (TM), Field Coordinator (FC), and quality assurance/quality control (QA/QC) coordinator. The TM is

responsible for project planning and coordination, production of project deliverables, and performance of the administrative tasks needed to ensure timely and successful completion of the project. The TM is also responsible for communicating with the Windward PM on progress of project tasks and any deviations from this technical memorandum. Significant deviations will be further reported to representatives of LDWG, EPA, and Ecology. As FC, Ms. Andersen will be responsible for managing field activities. As QA/QC coordinator, Ms. Andersen will be responsible for general field QA/QC oversight. She will ensure that samples are collected and documented appropriately, assist Dr. Kohn during imposex analysis, and coordinate with Columbia Analytical Services, Inc. (Columbia) if samples are to be archived (see below). Ms. Andersen can be reached as follows:

Helle Andersen
Windward Environmental LLC
200 W. Mercer St., Suite 401
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Facsimile: 206.217.0089
Email: hellea@windwardenv.com

If a sufficient number and distribution of gastropods are found during the pilot survey, they will be archived at Columbia and potentially analyzed for TBT (see Section 6.0). The laboratory PM at Columbia can be reached as follows:

Greg Salata
Columbia Analytical Services, Inc.
1317 So. 13th Avenue
Kelso, WA 98626
Telephone: 360. 577. 7222
Facsimile: 360. 425. 9096
Email: gsalata@kelso.caslab.com

Dr. Allan Fukuyama, an expert mollusk taxonomist, will verify the identifications of the gastropods collected in the LDW. Dr. Fukuyama can be reached as follows:

Allan Fukuyama
7019 157th St. SW
Edmonds, WA 98026
Telephone: 425.745.3349
Email: allanf@u.washington.edu

Dr Alan Kohn, professor emeritus at the University of Washington, will perform the imposex analysis of gastropods collected in the LDW. Dr. Kohn can be reached as follows:

Alan Kohn
Department of Zoology
University of Washington

Seattle, WA 98195
Telephone: 206.616.4383
Email: kohn@u.washington.edu

Kevin Li, an environmental scientist at the King County Environmental Laboratory, will supervise the deployment of the benthic sledge. Kevin Li can be reached as follows:

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Seattle, WA 98119
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3.0 Problem Definition/Background

The Phase 2 RI work plan (Windward 2004a) identified the need for a gastropod pilot survey to assess the feasibility and methods for collecting gastropod tissues so that TBT risks to benthic invertebrates in the LDW can be assessed. Of the benthic invertebrates that may inhabit the LDW, neo- and mesogastropods have been identified as particularly sensitive invertebrates to TBT (Meador et al. 2002). The most sensitive endpoint for gastropods is imposex.³ This section defines the issues and presents the background information and objectives for this pilot survey.

Relatively few data exist regarding the abundance and distribution of gastropods in the LDW, and the studies that do exist were not specifically designed to answer questions regarding the abundance and distribution of gastropods in the LDW (e.g., they may not have included sampling in areas with the most appropriate substrates; sampling devices may not have been the most appropriate for collecting gastropods). King County (1999) reported numerous gastropods (including both neo- and mesogastropods) in the lower reaches of the LDW, with the greatest abundance in the area between the southern tip of Harbor Island and Kellogg Island (river mile [RM] 0 to RM 0.5). At stations near Kellogg Island, Leon (1980) collected two individuals each of two different neogastropod species, *Nassarius* sp. and *Mitrella gouldii*, and one individual mesogastropod, *Barleeia* sp., using a 0.5-m² van Veen grab sampler. Williams (1990) collected a single unidentified mesogastropod larva using a 0.018-m² plankton pump in the subtidal area at the south end of Kellogg Island. No gastropods were reported in benthic community studies conducted by Cordell et al. (1996, 1997, 1999, 2001) or Ecology (2000). Table 3-1 presents the abundance of gastropods previously collected in the LDW, and Figure 3-1 (located in Section 10.0) shows the station locations of previous investigations of benthic invertebrate communities, including the stations where gastropods were found.

³ Imposex is defined as the development of male sexual characteristics in females

Table 3-1. Summary of existing data for gastropods collected in the LDW

ORDER	TAXON	ABUNDANCE (number of organisms)	MAXIMUM SHELL HEIGHT (cm)	ESTIMATED WEIGHT ^a (g)	LOCATION	RIVER MILE	SUBSTRATE	SAMPLING DEVICE	STUDY
Neogastropoda	<i>Nassarius</i> sp.	2	1.8-4.7	0.5-9.5	Stations 9 and 12	0.5 and 0.7	sandy mud	0.05-m ² grab	Leon 1980
	<i>Alia carinata</i>	1	1.0	0.09	DDS-3	0.4	81.2% fines	0.1-m ² grab	King County 1999
	<i>Mitrella gouldii</i> ^b	20	2.5	1.4	DDS-3, DDS-5, KI-1, KI-2	0.4-0.6	81.2-93.2% fines	0.1-m ² grab	King County 1999
		2	2.5	1.4	Stations 10 and 11	0.9	sandy mud	0.05-m ² grab	Leon 1980
Mesogastropoda	<i>Epitonium</i> sp.	98	1.5-3.2	0.3-3.0	DDS-3, DDS-5, KI-1, KI-2	0.4 – 0.5	81.2-93.2% fines	0.1-m ² grab	King County 1999
	<i>Mellanella</i> sp. ^c	1	na	na	DDS-3	0.4	81.2% fines	0.1-m ² grab	King County 1999
	<i>Alvania compacta</i>	30	0.3	0.002	DDS-1 DDS-3, DDS-5, KI-1, KI-2	0.4 – 0.5	14.6 (DDS-1) and 81.2-93.2% fines	0.1-m ² grab	King County 1999
	<i>Barleeia</i> sp.	1	0.3-0.4	0.002-0.01	Station 12	0.7	mud with wood chips	0.05-m ² grab	Leon 1980
	<i>Tachyrhynchus</i> sp.	1	2.0-3.0	0.7-2.4	DDS-3	0.4	81.2% fines	0.1-m ² grab	King County 1999
	Unidentified larva	1	na	na	south end Kellogg Island	0.9	very fine sediment	0.018-m ² area plankton pump	Williams 1990
^d Opisthobranchia	<i>Odostomia</i> sp.	4	0.6-1.0	0.02-0.5	DDS-3, DDS-5, KI-2	0.4 – 0.5	81.2-93.2% fines	0.1-m ² grab	King County 1999
Nudibranchia	Aeolidacea	1	na	na	DDS-3	0.4	81.2% fines	0.1-m ² grab	King County 1999
Cephalaspidea	<i>Gastropterion pacificum</i>	10	na	na	KI-1, KI-2	0.5	90.6-93.2% fines	0.1-m ² grab	King County 1999
	<i>Melanochlamys diomedea</i>	9	na	na	DDS-5, KI-1, KI-2	0.4 – 0.5	85.2-93.2% fines	0.1-m ² grab	King County 1999

^a Estimated total weight of tissue (shell excluded) for all individuals collected, based on an article by Tokeshi et al. (2000) and a gastropod tissue moisture content of 80%

^b Also known as *Nitidella gouldi*

^c Small parasite on sea cucumbers

^d Subclass of Mollusca; other taxa in this column are orders of Mollusca

na – not available

Also, few data exist regarding TBT concentrations in benthic invertebrate tissues collected from the LDW. The only TBT data available for benthic invertebrate species in the LDW are from four composite tissue samples of amphipods (mixed *Corophium* and *Eogammarus* sp.) collected near Kellogg Island, with associated sediment samples (King County 1999).

Thus, overall, there are few data characterizing site use of the LDW by gastropods, and few tissue data are available regarding the concentration of TBT in benthic invertebrates in the LDW. Therefore, the objectives of this gastropod pilot survey are:

- ◆ to determine the presence and general distribution of gastropods (particularly neo- and mesogastropods) in the LDW
- ◆ to assess the feasibility of collecting a sufficient number of gastropods (or a surrogate) for TBT tissue analysis

Information obtained from the gastropod pilot survey will be used to determine which of three possible hypothesized scenarios are present in the LDW: A) very few gastropods utilize the LDW, B) some gastropods are present, or C) gastropods are abundant in the LDW (Table 3-2). The scenario supported by the pilot survey data will determine which organisms will be chemically analyzed for TBT, and which toxicological endpoints are relevant for benthic invertebrates in the Phase 2 ERA. These decisions will be made in consultation with EPA and Ecology, as discussed in Section 1.0.

Table 3-2. Potential gastropod scenarios in the LDW

SCENARIO ^a	FIELD RESULTS	ORGANISMS TO ANALYZE FOR TBT	RELEVANT ENDPOINTS
A	very few gastropods found throughout habitat types and over the range of TBT concentrations in sediment	market basket benthic invertebrates	survival, growth, and reproduction (not including imposex)
B	gastropods found, but insufficient tissue mass available to analyze for TBT	potential collection of a surrogate taxon or group ^b	survival, growth, and reproduction (including imposex)
C	gastropods are found and are collected in sufficient abundance to analyze for TBT	gastropods	survival, growth, and reproduction (including imposex)

^a The means to distinguish the most appropriate scenario (or combination of scenarios) will be determined in consultation with EPA and Ecology

^b The determination of the suitability and feasibility of collecting and analyzing a surrogate taxon or group will be made in consultation with EPA and Ecology based on the results of this pilot survey.

4.0 Project/Task Description and Schedule

To address the data needs and study objectives identified in Section 3.0, a pilot survey will be conducted June 16 to 18, 2004. The results of this pilot survey will be used to determine which scenario presented in Table 3-2 best describes the use of the

LDW by gastropods, to assess the feasibility of collecting enough gastropods for chemical analysis, and to evaluate which collection methods are most suitable for the August 2004 sampling event. As discussed in Sections 6 and 7, gastropods collected during the pilot survey will be identified and evaluated for imposex. These gastropods may also be archived for chemical analyses depending on the number, mass, and distribution of gastropods found.⁴

A technical memorandum presenting the results of the survey, identification, and imposex analyses will be submitted on July 7, 2004, and a meeting with EPA and Ecology is tentatively scheduled for mid July 2004 to discuss the data presented in the memo. The outcome of the pilot survey and decisions reached during the meeting will guide the study design that will be used to collect co-located tissue and sediment data according to the final version of the benthic invertebrate QAPP (Windward 2004a). Collection of gastropods (and other benthic invertebrates) and co-located sediment for TBT analysis will occur in August 2004.

5.0 Data Generation and Acquisition

This section describes the methods that will be used during the pilot survey. The pilot survey will include both intertidal and subtidal areas in the lower four miles (i.e., RM 0.0 to 4.0) of the LDW. This area will be targeted because the full range of TBT sediment concentrations in the LDW can be found within this area and because previous studies (Table 3-1) have found gastropods only in the lower, more saline portion of the waterway. The survey will also cover a large range of habitats, from mud flats to rocky areas, to ensure that potential gastropod habitats have been surveyed. This section presents detailed sampling methods for intertidal and subtidal areas in Sections 5.1 and 5.2, respectively, the identification scheme (Section 5.3), and field equipment (Section 5.4).

5.1 INTERTIDAL SAMPLING DESIGN AND METHODS

This section presents the sampling design and methods for the intertidal survey.

5.1.1 Intertidal sampling design

Intertidal areas were selected based on the following three criteria:

- ◆ to provide spatial coverage within the LDW and survey a large range of potential habitats, particularly in the downstream, more saline areas where gastropods have been found in past studies
- ◆ to cover the range of TBT concentrations in intertidal surface sediment, to the extent indicated by historical samples

⁴ The decision whether to analyze these gastropods for TBT will be made in consultation with EPA and Ecology. Co-located sediment will not be collected during the pilot survey, as discussed in Section 5.1.2.

- ◆ to resample areas where gastropods have been found in past surveys (Table 3-1)

Survey locations were placed in nine intertidal areas from RM 0.1 to RM 2.4, in both high and medium salinity environments (see Appendix D of the benthic invertebrate QAPP [Windward 2004a]). Surveys will be conducted for approximately two hours before and after a negative tide⁵ to facilitate the search for organisms. Within these areas, a large range of potential habitats will be surveyed. The selected intertidal areas include sandy and muddy substrate, rocky areas, and seawalls. In the search for gastropods, rocks will be overturned, sand/mud flats and seawalls will be surveyed, and attached algae will be examined. Selected locations are shown in Figure 5-1 (located in Section 10.0).

These areas were also selected to cover the range of TBT concentrations in intertidal surface sediment, to the extent indicated by historical samples. Figure 5-2 illustrates the range of TBT concentrations in LDW sediment as a function of cumulative area, and also shows the selected intertidal and subtidal survey locations. The selected intertidal areas represent a range of TBT concentrations from 31 to 216 µg/kg dry weight (dw) (approximately 41st to 91st percentile concentrations), based on the limited intertidal surface sediment data available. Selected intertidal survey areas and corresponding TBT concentrations in sediment are presented in Table 5-1.

One intertidal area, G2a, was placed near the Kellogg Island sampling station where a single unidentified mesogastropod larva was observed in Williams (1990). No other gastropods have been reported from any intertidal areas in the LDW, including those conducted by Cordell et al (1996, 1997, 1999, 2001). However, the sampling gear used by Cordell et al. only sampled a small surface area (0.0024-m²) resulting in a lower probability of collecting gastropods, which are mobile epibenthic organisms that vary in size.

⁵ June 16th low tide at 11 am; survey time 9 am – 1 pm
June 17th low tide at 11:32 am; survey time 9:30 am – 1:30 pm
June 18th low tide at 12:06 pm; survey time 10 am – 2 pm.

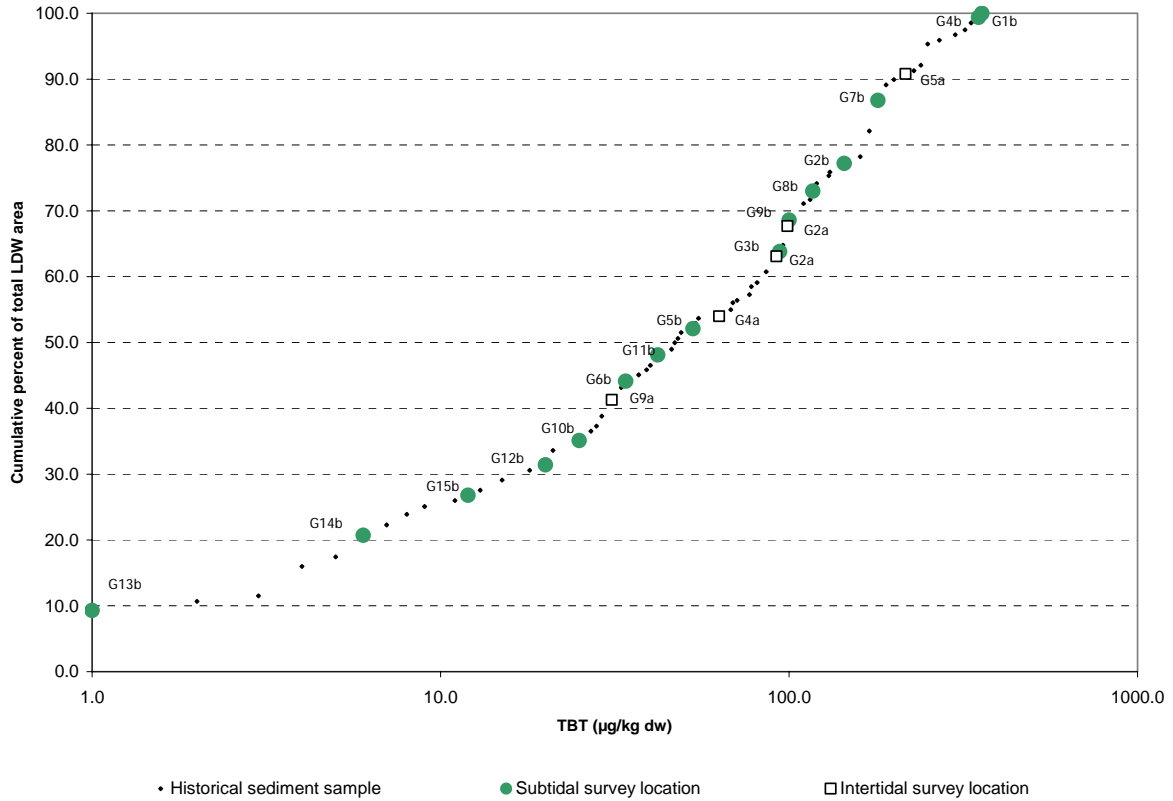


Figure 5-2. Cumulative frequency distribution of historical surface sediment TBT concentrations in the LDW and gastropod pilot survey locations

Note: TBT concentrations in intertidal surface sediment were only available from five of the nine pilot survey locations, two of which are in the area west of Kellogg Island. The other four intertidal locations are not depicted because historical data are not available from those locations.

Table 5-1. Intertidal gastropod pilot survey locations

ID	DAY	LOCATION	RIVER MILE	TBT
				SEDIMENT CONCENTRATION (µg/kg dw)
G1a	1	North of Kellogg Island	0.1 – 0.2	no data
G2a	2	West of Kellogg Island	0.5 – 0.9	92 and 99 ^a
G3a	1	East of Kellogg island	0.7 – 0.9	no data
G4a	1	East of Kellogg Island	0.6 – 0.9	63
G5a	2	West bank	1.4 – 1.5	216
G6a	2	East bank	1.4 – 1.6	no data
G7a	3	Slip 2	1.7 – 1.9	no data
G8a	3	West of Slip 3	2.0 – 2.1	no data
G9a	3	South of Slip 3	2.2 – 2.4	31

^a Two surface sediment samples from intertidal area G2a were analyzed for TBT during past surveys.

5.1.2 Intertidal sampling methods

The gastropod pilot survey will be conducted over three days. Three intertidal areas will be surveyed each day, starting with the downstream areas and moving upstream. Each intertidal area will be divided into 100-m segments parallel to the waterline, with a maximum of four segments per intertidal area (e.g., large intertidal areas, such as G2a, will be divided into four segments totaling 400 m). Each area will also be divided into three sections perpendicular to the waterline up to mean higher high water (MHHW). A team of three field crew members will search each intertidal area; one person will walk along the waterline, one person will walk midway up the beach, and the third person will walk near MHHW. Crew members will cover 100 m at their designated elevation for a set amount of time, depending on the number of gastropods found (15 to 30 minutes per 100-m segment). By performing the survey over a set time and distance, a general catch per unit effort (CPUE) can be estimated for the nine intertidal areas. The CPUE information will be used to develop a general estimate of the level of effort that will be needed for the collection of the gastropod tissue samples and co-located sediment samples in August. Survey locations will be documented with global positioning system (GPS) measurements at the start and end of each segment, and at locations within a segment where gastropods are collected if they are not evenly distributed.

All individual gastropods found within each 100-m segment will be rinsed with distilled water, placed together in a wide-mouth glass jar quarter filled with LDW water,⁶ and stored on ice. A maximum of four sampling jars with gastropods will be collected per intertidal area and taken to Windward for further identification (see Section 7.0). The number of gastropods per sampling effort and the sampling locations using GPS will be noted on the field form (see Appendix A). All gastropods will be photographed in groups by species or genera, returned to the sampling jar, and kept in a refrigerator at Windward,⁷ as described in Section 7.2, until the imposex analysis is performed on June 21, 2004. Following the imposex analysis, gastropod samples may be sent to Columbia Analytical Services for potential chemical analysis if the minimum weight requirement of 2 g of neo- or mesogastropod tissue (not including the shell) is achieved.

The decision whether to archive these samples for chemical analyses will be made in consultation with EPA and Ecology immediately following the pilot survey. While collection of tissue for chemical analyses is not the objective of this pilot study, archiving collected samples may be viewed as cost-effective depending on the level of effort required to collect these samples. If the samples are archived, they will be shipped to Columbia as described in Section 6.4, and analyzed in accordance with

⁶ Quarter filling jars with gastropods with LDW water and storing them in the refrigerator was recommended by Dr. Alan Kohn to keep gastropods alive until they are assessed for imposex on June 21, 2004.

⁷ The lids of the jars will be off in the refrigerator to allow air exchange for the live gastropods. A mesh will cover the opening the jars to contain the gastropods.

the methods and protocols outlined in the benthic invertebrate QAPP (Windward 2004a). Co-located sediment samples will not be collected during this pilot gastropod survey because the results of this survey will be used to determine the most appropriate sampling techniques and sampling locations for the August fieldwork.

During the intertidal survey of the 100-m segments, other candidate invertebrates will be collected in a separate jar to assess the feasibility of collecting surrogate species (Scenario B in Table 3-2). The collected invertebrates will be photographed as a group and returned to each survey area before traveling to the next area. After completing the gastropod search, the abundance of infaunal surrogate species will be assessed by placing a 0.1-m² stainless steel transect frame randomly at five locations between mean lower low water (MLLW) and mean higher high water (MHHW) within the first 100-m segment of the intertidal area. The invertebrates in the sediment will be collected by digging the sediment from the frame to a depth of 10 cm and transferring it to a 1.0-mm mesh sieve. The sediment in the sieve will be broken up with a gentle spray of LDW water using a spray bottle and rinsed to separate the organisms from sediment and organic matter. All organisms remaining in the sieve will be sorted by major taxonomic group: Annelida, Crustacea, Mollusca, Echinodermata, and miscellaneous phyla, photographed, and returned to the digging site. Field equipment for the intertidal surveys is described in Section 5.4, and decontamination procedures are discussed in Section 6.2.

5.2 SUBTIDAL SAMPLING DESIGN AND METHODS

This section presents the sampling design and methods for the subtidal survey.

5.2.1 Subtidal sampling design

The subtidal survey locations were selected based on the following three criteria:

- ◆ to provide spatial coverage within the LDW and survey a large range of potential habitats, particularly in the downstream, more saline areas
- ◆ to cover the range of TBT concentrations in subtidal surface sediment, to the extent indicated by historical samples
- ◆ to resample areas where gastropods have been found in past surveys (Table 3-1)

Survey locations were placed in 15 subtidal areas between RM 0.0 and RM 3.8 in high salinity environments within the LDW (see Appendix D of the benthic invertebrate QAPP for determination of the salinities [Windward 2004a]). Within these areas, a large range of potential habitats will be surveyed, as shown by the physical parameters presented in Table 5-2. Subtidal hard substrate present in the LDW will not be sampled. However, the survey of the intertidal areas at negative tides in mid June will include a survey of any hard substrate (e.g., riprap) along the waters edge at the intertidal locations shown in Figure 5-1.

Table 5-2. Habitat information for the subtidal gastropod pilot survey locations

ID	LOCATION	RIVER MILE	DEPTH (-FT MLLW)	% FINES	% TOC	ESTIMATED % TIME <5 PPT
G1b	south of southern tip of Harbor Island	0.0	26.8	48	2.33	0
G2b	south of southern tip of Harbor Island	0.1	39.6	74	1.89	0
G3b	south of southern tip of Harbor Island	0.1	12.4	25	1.72	0
G4b	north of Duwamish Diagonal outfall	0.3	39.8	50	2.06	0
G5b	north of Duwamish Diagonal outfall	0.3	22.3	24	0.82	0
G6b	near Duwamish Diagonal outfall	0.5	34.2	42	1.78	0
G7b	south of Duwamish Diagonal outfall	0.6	26.7	48	2.49	0
G8b	south of Duwamish Diagonal outfall	0.6	17.0	21	na	0
G9b	Slip 1	0.9	27.9	28	na	0
G10b	north of Slip 2	1.6	22.7	45	0.7	0
G11b	mouth of Slip 2	1.8	17.4	25	1.82	0
G12b	west of slip 3	2.0	13.9	13	0.76	0
G13b	south of West Michigan outfall	2.4	6.7	21	0.09	6.0
G14b	mouth of Slip 4	2.8	16.8	36	1.8	0.31
G15b	south of T117	3.8	4.0	19	1.45	72.8

ppt – parts per thousand

na – not available

The selected subtidal locations represent a range of depths (-4 to -40 ft MLLW), % fines (13-74%), total organic carbon (TOC) (0.09 – 2.5%), and salinities (0 to 73% of the time below 5 ppt).⁸ The selected subtidal locations also represent a range of TBT concentrations from 1 to 358 µg/kg dw (approximately 9th to 100th percentile concentrations), based on the available Phase 1 surface sediment data, as shown in Figure 5-1 and Table 5-3. Higher TBT concentrations are generally found in the downstream segment of the waterway (RM 0-1), with concentrations ranging from 34 to 358 µg/kg dw. Upstream of RM 3.5, TBT concentrations in sediment decreased to 20 µg/kg dw or less, except for two locations (Slip 6 and RM 3.8), where concentrations were as high as 50 µg/kg dw.

Table 5-3. TBT concentrations at the subtidal gastropod pilot survey locations

ID	DAY	LOCATION	RIVER MILE	TBT ^a	
				SED CONC (µg/kg dw)	% OF AREA ^b
G1b	1	south of southern tip of Harbor Island	0.0	358	100
G2b	1	south of southern tip of Harbor Island	0.1	144	77.2
G3b	1	south of southern tip of Harbor Island	0.1	94	63.8
G4b	1	north of Duwamish Diagonal outfall	0.3	350	99.4
G5b	1	north of Duwamish Diagonal outfall	0.3	53	52.1

⁸ See Appendix D of the benthic invertebrate QAPP for an explanation of the salinity regimes.

ID	DAY	LOCATION	RIVER MILE	TBT ^a	
				SED CONC (µg/kg dw)	% OF AREA ^b
G6b	2	near Duwamish Diagonal outfall	0.5	34	44.1
G7b	2	south of Duwamish Diagonal outfall	0.6	180	86.8
G8b	2	south of Duwamish Diagonal outfall	0.6	117	73.0
G9b	2	Slip 1	0.9	100	68.6
G10b	2	north of Slip 2	1.6	25	35.1
G11b	3	mouth of Slip 2	1.8	42	48.1
G12b	3	west of slip 3	2.0	20	31.4
G13b	3	south of West Michigan outfall	2.4	1	9.3
G14b ^c	3	mouth of Slip 4	2.8	6	20.7
G15b ^c	3	south of T117	3.8	12	26.8

^a The coordinates for the existing sampling stations with TBT concentrations in sediment are listed in Table 5-4

^b Cumulative percent of LDW subtidal area with chemical concentrations below those shown

^c These locations may not be surveyed if gastropods are not found at Locations G10b, G11b, G12b, or G13b and time is limited. EPA and Ecology would be consulted prior to dropping these two stations on the third day of the survey.

Three studies have collected gastropods at nine subtidal stations in the LDW within the last 14 years. The most recent study (King County 1999) collected gastropods at five stations, two of which have recently been dredged (DDS-1 and DDS-3). Subtidal locations have been placed near the remaining three sampling stations (KI-1, KI-2, and DDS-5). Three additional survey locations are placed near historical sampling stations (Leon 1980), as follows:

- ◆ subtidal location G6b was placed near DDS-5 and KI-1 (King County 1999)
- ◆ subtidal location G7b was placed near DDS-5 (King County 1999)
- ◆ subtidal location G8b was placed near KI-2 (King County 1999) and station 9 (Leon 1980)

Coordinates of all gastropod pilot survey sampling locations, and their associations with historical sampling locations, are shown in Table 5-4.

Table 5-4. Target coordinates of each gastropod pilot survey location

LOCATION ID	EASTING (x)	NORTHING (y)	HISTORICAL SAMPLE IDENTIFICATION ^a		
			EVENT ID	LOCATION ID	SAMPLE ID
G1b	1266882.588	211229.233	Harbor Island RI	543	K-07
G2b	1266643.732	210653.679	EPA SI	622	SD-DR056-0000
G3b	1266066.595	210603.225	EPA SI	599	SD-DR033-0000
G4b	1266550.578	209883.238	Harbor Island RI	540	K-04-B
G5b	1266284.687	209905.740	EPA SI	633	SD-DR067-0000
G6b	1266815.320	208950.778	EPA SI	647	SD-DR081-0000
G7b	1267131.379	208314.210	EPA SI	578	SD-DR011-0000
G8b	1266636.292	208454.731	PSAMP/NOAA98	1162	203

LOCATION ID	EASTING (X)	NORTHING (Y)	HISTORICAL SAMPLE IDENTIFICATION ^a		
			EVENT ID	LOCATION ID	SAMPLE ID
G9b	1268178.985	206710.675	EPA SI	585	SD-DR018-0000
G10b	1268746.730	203539.686	EPA SI	658	SD-DR092-0000
G11b	1269108.039	202682.355	EPA SI	667	SD-DR101-0000A
G12b	1269407.612	201508.020	EPA SI	699	SD-DR133-0000
G13b	1270775.744	199947.031	EPA SI	706	SD-DR140-0000
G14b	1272793.822	198480.985	EPA SI	749	SD-DR183-0000
G15b	1275822.018	194661.846	EPA SI	776	SD-DR210-0000

^a The target coordinates for the gastropod pilot survey sampling locations are identical to coordinates of previously sampled locations identified here by event, location, and sample ID.

5.2.2 Subtidal sampling methods

The gastropod pilot survey will be conducted over three days. Five subtidal locations will be surveyed each day, starting with the stations farthest downstream. On the first day of the pilot survey, the performance of the preferred sampling device, a benthic sledge sampler (Figure 5-3), will be assessed to determine whether it is reliable and retains relevant samples of organisms from the surface of the sediment. The benthic sledge is preferred because it is designed to collect epibenthic organisms, such as gastropods. The assessment of the benthic sledge will include ease of operation (i.e., whether the sledge gets stuck or lodged too deeply in the sediment or skims the surface of the sediment), performance of tows of similar length and duration, and ability to collect epibenthic organisms. Test runs will be performed under the supervision of Kevin Li (who has considerable experience with the sledge) before positioning the sampling vessel over the first subtidal location. During these test runs, the scope of the tow rope and the deployment time of the sledge will be determined. After the test runs, the benthic sledge will be deployed at five subtidal locations located between RM 0 and RM 0.9 on day 1 of the pilot survey.

The benthic sledge consists of a metal frame 20 cm high by 56 cm wide. A bag (approximately 50 cm by 50 cm) with a 1-mm mesh is attached to the back of the frame and protected by heavy canvas cloth. Two V-shaped brackets are attached to the corners of the sledge and linked together by a chain 86 cm long. A rope 1 cm in diameter is attached to the center of the chain and the sledge is pulled by a boat. The tow rope will be adjusted so that it maintains a scope of approximately 1:5 (1 m depth to 5 m of rope let out). The scope may be adjusted depending on whether or how far the sledge digs into the sediment. At each location, the sledge will be pulled over the bottom at the same speed and for the same amount of time. GPS will be used to identify the start and end of each sledge deployment.

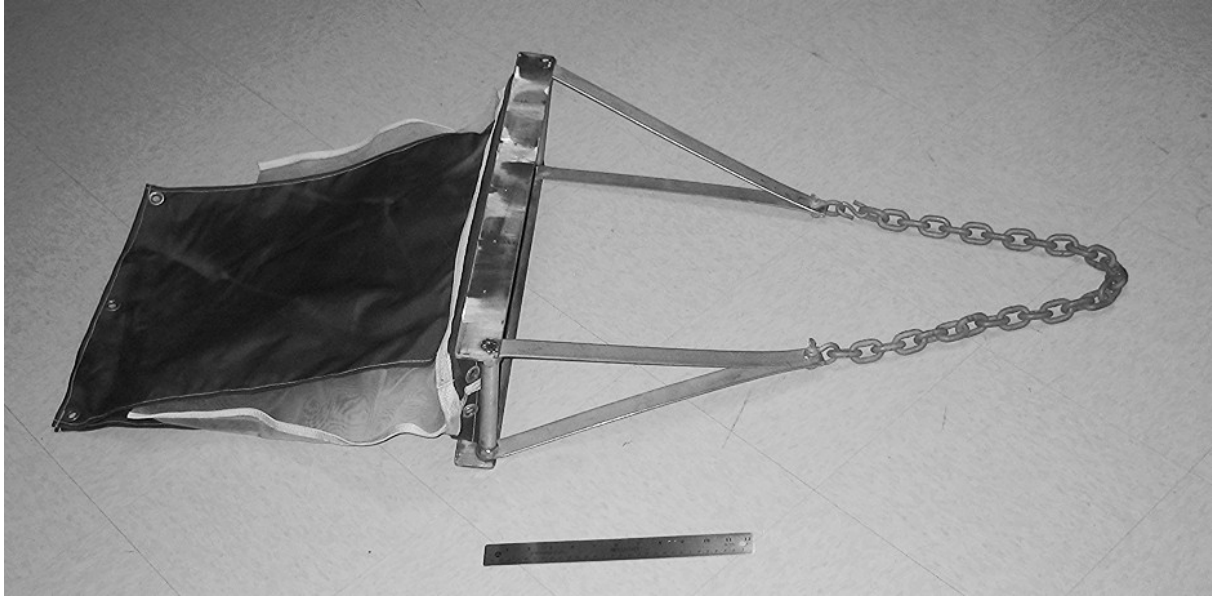


Figure 5-3. Benthic sledge sampler

At the completion of the designated pulling time (as determined in the field trials), the sledge will slowly be winched out of the water and into the boat. The contents in the bag will be rinsed into a 1.0-mm mesh sieve, and the contents will be sorted into major taxonomic groups (Annelida, Crustacea, Mollusca, Echinodermata, and miscellaneous phyla), and photographed to document the abundance of potential surrogate species. Any gastropods in the sieve will be retained and the number of gastropods per sampling effort will be noted on the field collection form (see Appendix A). The gastropods from each tow will be rinsed with distilled water, placed into a wide-mouth glass jar quarter filled with Elliott Bay surface water,⁹ and stored on ice. The sampling process will be performed five times within the general vicinity of each GPS location to a distance of approximately 10-50 m¹⁰ to get an estimate of the catch variability between the tows of the sledge. This information will be used to derive an estimate of the abundance of gastropods at each location, and to estimate the time and sampling level of effort that will be required for the gastropod (or surrogate) tissue and co-located sediment collection in August.

If the sledge works well the first day, it will be used the remaining two days of the pilot survey. If the sledge is unsuccessful at collecting gastropods or too difficult to operate, the remainder of the pilot survey will be conducted using a double van Veen grab sampler (two ganged van Veen samplers, each 0.1 m²). If the van Veen grab sampler is substituted for the sledge, the subtidal locations surveyed using the sledge on the first day will be re-occupied within 10 m, and these locations will be

⁹ Surface water will be obtained from Elliott Bay so that it has a salinity closer to that in subtidal environments than surface water obtained from the LDW at the sampling area.

¹⁰ The tow length will be determined in the field based on the test runs. An effort will be made to keep the tows as short as possible to facilitate subsequent collection of co-located tissue and sediment samples. The same exact segment will not be towed more than once.

resampled with the van Veen grab sampler. The deployment of the van Veen grab sampler is described in steps listed below.

1. Maneuver the sampling vessel to the pre-identified sampling location (within 1-2 m of the intended location) using GPS. The GPS is located on the winch arm right over the grab sampler.
2. Open the grab sampler jaws into the deployment position.
3. Guide the sampler overboard until it is clear of the vessel.
4. Lower the sampler through the water column to the bottom at approximately 0.3 m/s.
5. Record the GPS location of the boat when the sampler reaches bottom.
6. Retrieve the sampler and raise it at approximately 0.3 m/s.
7. Guide the sampler aboard the vessel and place it on the work stand on the deck; using care to avoid jostling that might disturb the integrity of the sample.
8. Examine the sample using the following sediment acceptance criteria:
 - ◆ Sediment is not extruded from the upper face of the sampler such that organisms may have been lost
 - ◆ Overlying water is present (indicating minimal leakage)
 - ◆ The sediment surface is relatively flat (indicating minimal disturbance or winnowing)
 - ◆ The entire surface of the sample is included in the sampler
 - ◆ The following penetration depths are achieved at a minimum:
 - 4-5 cm for medium-coarse sand
 - 6-7 cm for fine sand
 - ≥ 10 cm for muddy sediment

If these sample acceptance criteria are not achieved, the sample will be rejected.

After sample acceptance, the following observations will be noted on the field collection form (see Appendix A):

- ◆ station GPS location
- ◆ depth as read by the boat's depth sounder
- ◆ gross characteristics of the surficial sediment including texture, color, biological structures, odor, presence of debris and oily sheen
- ◆ gross characteristics of the vertical profile, i.e., changes in sediment characteristics and redox layer if visible
- ◆ maximum penetration depth (nearest 0.5 cm)

- ◆ comments relative to sample quality

The contents of the grab sampler will be transferred directly to a 1.0-mm mesh sieve, and gently rinsed using a spray bottle using LDW water to separate the organisms from sediment and organic matter. Any gastropods retained in the sieve will be collected as described above, and the number of gastropods per sampling effort will be noted on the field collection form (see Appendix A). The remaining organisms will be separated into the major taxonomic groups Annelida, Crustacea, Mollusca, Echinodermata, and miscellaneous phyla; photographed; and returned to the sampling site. The van Veen grab sampler will be deployed five times within 10 m of each location to get an estimate of the catch variability between the grabs. This information will be used to estimate the abundance of gastropods at each location, and to estimate the time and sampling level of effort that may be needed for the gastropod (or surrogate) tissue and co-located sediment collection in August. Field equipment for the subtidal surveys is listed in Section 5.4, and decontamination procedures are discussed in Section 6.2.

5.3 SAMPLE IDENTIFICATION SCHEME

Each sampling location will be assigned a unique alphanumeric location identification number. The first three letters of the location identification number are "LDW" to identify the Lower Duwamish Waterway project area. The next character indicates the location. The location naming convention for this pilot survey uses sequential numbers starting with 1, prefaced by a single letter to indicate the type of sampling location. The 24 gastropod sampling locations are divided into intertidal and subtidal groups of 9 and 15, respectively. Each group is numbered independently. The intertidal locations are numbered G1a (northernmost) to G9a (southernmost). The subtidal locations are numbered G1b (northernmost) to G15b (southernmost). Sample type will be indicated with a T suffix for tissue and an S suffix for survey. The last character will identify the segment surveyed (1 through 4) or the tow/grab number (1 through 5). The composite gastropod tissue sample collected at the intertidal location G7a in the first 100-m segment would thus be identified as LDW-G7a-TS-1, and the composite gastropod tissue sample collected at subtidal location G1b during the second 100-segment would be identified as LDW-G1b-TS-2.

5.4 FIELD EQUIPMENT

Field equipment needs for the gastropod pilot survey are listed in Table 5-4. Prior to mobilization, this list will be consulted to ensure all equipment is available and pre-cleaned. As part of the mobilization process, each item will be double checked by the FC.

Table 5-5. Field equipment needs for the gastropod pilot survey

benthic sledge	coolers	field notebooks
double 0.1-m ² van Veen grab sampler	wet ice	pens/pencils/Sharpies
0.1-m ² stainless steel transect frame	handheld GPS	tide tables
0.5-mm mesh sieves	digital camera	technical memorandum
spray bottle	batteries	transect tape
stainless steel shovel	powder-free nitrile exam gloves	paper towels
Alconox [®] detergent	rubber work gloves	field sample sheets
scrub brushes	first aid kit	study area maps
distilled water	duct tape	chain-of-custody forms
wide-mouth glass jars	boots (or waders)	personal flotation devices

6.0 Sample Handling and Custody Requirements

If found, gastropods will be collected during the pilot survey for three purposes:

- ◆ to be definitively identified at Windward (see Section 7.0)
- ◆ imposex analysis
- ◆ for potential archiving at Columbia for TBT analysis if a sufficient number and distribution of gastropods is collected (see Section 5.1.2)

In the event that a sufficient number and distribution of gastropods are collected in June and analyzed for TBT, co-located composite sediment samples will be collected in early August at the collection locations. The methods for determining how best to collect co-located sediment samples will be described in the approved benthic invertebrate QAPP prior to sediment collection.

Sample custody is a critical aspect of environmental investigations. Sample possession and handling must be traceable from the time of sample collection, through laboratory and data analysis, to delivery of the sample results to the recipient. This section describes the minimum project requirements for sample handling and custody procedures.

6.1 SAMPLE HANDLING PROCEDURES

All gastropods collected at each subtidal location or 100-m segment of intertidal area will be placed in appropriate-sized, certified-clean, wide-mouth glass jars containing LDW (intertidal samples) or Elliott Bay (subtidal samples) surface water, and capped with Teflon[®]-lined lids.

Sample labels will be waterproof and self-adhering. Each sample label will contain the project number, sample identification, and initials of the person(s) collecting the sample. A completed sample label will be affixed to each sample container. The labels will be covered with clear tape immediately after they are affixed to the container to protect them from being stained or spoiled from water and sediment.

The samples will be kept on ice in the field and during transport to Windward. After identification at Windward, the gastropods will be kept as described in Section 7.2 until imposex analysis is performed on June 21, 2004. Following the imposex analysis, gastropod samples may be shipped frozen to Columbia for potential chemical analyses (see Section 5.1.2). The samples will be frozen at the laboratory and kept until further notice from LDWG.

6.2 DECONTAMINATION PROCEDURES

Care will be taken in the field to avoid contaminating the gastropods during sampling. The field crew will wear nitrile powder-free examination gloves. A new pair will be worn at each sampling area or location.

The benthic sledge, 0.5-mm sieve, and the van Veen grab sampler are the only equipment that will be used for gastropod collection. All equipment touching the gastropod samples will be decontaminated following PSEP (1997) guidelines between areas or locations:

1. Rinse with site water and wash with scrub brush until free of sediment
2. Wash with phosphate-free detergent
3. Rinse with site water
4. Rinse with distilled water

The rinse and wash water will be disposed of at the site. Acid or solvent washes will not be used in the field because of safety considerations and problems associated with rinsate disposal and sample integrity. Specifically:

- ◆ The use of acids or organic solvents may pose a safety hazard to the field crew
- ◆ Disposal and spillage of acids and solvents during field activities pose an environmental concern
- ◆ Residues of solvents and acids on sampling equipment may affect sample integrity for chemical testing

Any sampling equipment that cannot be cleaned to the satisfaction of the FS will not be used for further sampling activity.

6.3 SAMPLE CUSTODY PROCEDURES

Samples are considered to be in custody if they are: 1) in the custodian's possession or view, 2) retained in a secured place (under lock) with restricted access, or 3) placed in a container and secured with an official seal(s) such that the sample cannot be reached without breaking the seal(s). Custody procedures will be used during collection, transport, and storage of the gastropod samples. Custody procedures will be initiated during sample collection, and a chain-of-custody (COC) form will accompany the samples throughout the process. Each person who has custody of the

samples will sign the COC form and ensure that the samples are not left unattended unless properly secured. Minimum documentation of sample handling and custody will include:

- ◆ sample location, project name, and unique sample number
- ◆ sample collection date and time
- ◆ any special notations on sample characteristics or problems
- ◆ initials of the person collecting the sample
- ◆ date sample was sent to each laboratory
- ◆ shipping company name and waybill number

The FC will be responsible for all sampling tracking and custody procedures for samples in the field. The FC will be responsible for final sample inventory and will maintain sample custody documentation. The FC will also complete COC forms prior to removing samples from the sampling area. At the end of each day, and prior to transfer, COC entries will be made for all samples. Information on the labels will be checked against sample log entries, and sample tracking forms and samples will be recounted. COC forms will accompany all samples, and signed at each point of transfer. Copies of all COC forms will be retained and included as an appendix to any data report associated with the chemical analysis of these samples.

6.4 SHIPPING REQUIREMENTS

The results of Dr. Kohn's analyses and the distribution and abundance of gastropods collected will be considered in deciding whether to ship samples to Columbia for archiving, in consultation with EPA and Ecology. All procedures and methods will be in accordance with the benthic invertebrate QAPP (Windward 2004a). Prior to shipping, sample containers will be wrapped in bubble wrap and securely packed inside a cooler with frozen blue ice packs or crushed ice. The original signed COC forms will be placed in a sealable plastic bag, sealed, and taped to the inside lid of the cooler. Fiber tape will be wrapped completely around the cooler. On each side of the cooler a *This Side Up* arrow label will be attached; a *Handle with Care* label will be attached to the top of the cooler, and the cooler will be sealed with a custody seal in two locations.

The laboratory will ensure that COC forms are properly signed upon receipt of the samples, and will note questions or observations concerning sample integrity on the COC records. The Laboratory PM will contact the TM immediately if discrepancies between the COC forms and the sample shipment are discovered upon receipt. The Laboratory PM will specifically note any coolers that do not contain ice packs or that are not sufficiently cold ($4 \pm 2^{\circ}\text{C}$) upon receipt. At Columbia, each sample will be assigned a unique laboratory number, and samples will be grouped in appropriate sample delivery groups. All samples will be handled so as to prevent contamination

or loss of any sample. Samples will be assigned a specific storage area within the laboratory, and will be kept there until compositing instructions are received.

7.0 Taxonomic Identification Methods and Imposex Analysis

7.1 TAXONOMIC IDENTIFICATION METHODS

If gastropods are found during the pilot survey, they will be retained as described in Sections 5.1.2 and 5.2.2. Prior to June 21, 2004, the gastropods collected will be identified to order, and if possible to genus or species at the Windward laboratory, and common taxonomic keys will be used and a reference collection will be made of all gastropod taxa. The reference collection created by Windward will be verified by Dr. Allan Fukuyama, an expert mollusk taxonomist. If any discrepancies are identified, a resolution will be reached between Dr. Fukuyama and Windward on the proper identification(s) and any inconsistency will be corrected throughout the data set.

Samples will be handled so as to avoid touching the tissue inside the shell. Each gastropod will be weighed in the shell (wet weight to nearest 0.1 g), and the height of the shell will be measured. These two measurements will aid in estimating the collected tissue mass at each location (Tokeshi et al. 2000). After the gastropods have been identified and measured, they will be returned to the sampling jars and stored as described in Section 7.2 at Windward until imposex analysis is performed on June 21, 2004.

7.2 IMPOSEX ANALYSIS

After identification, the gastropods will be returned to their original sample jars containing LDW water (for gastropods collected from the intertidal zone) or Elliott Bay surface water (for gastropods collected from the subtidal zone), and stored in a refrigerator (with mesh covering the jars) until June 21, 2004, when they will be delivered by Windward to Dr. Kohn's laboratory at the University of Washington.

The imposex analysis will be performed by Dr. Kohn. First, the shells will be cracked using a small hammer and the gender of each gastropod will be determined. The males will be returned to new, precleaned, and labeled sampling jars and stored in coolers with ice. The females will be examined for imposex using the vas deferens sequence (VDS) index (Spence et al. 1990). This index consists of six stages describing the development of vas deferens from its initial appearance and growth (stage 1-4) to overgrowth of the genital papilla occluding the vulva (stage 5) resulting in a build up of egg capsules (stage 6). Stages 5 and 6 render the female effectively sterile. After examination, female gastropods will be placed in separate vials¹¹ and stored on ice

¹¹ Female gastropods will be placed in jars either individually or in groups, depending on where the gastropods were collected, the results of the imposex analyses, and the mass of the gastropods. The FC will communicate with the PM to make these decisions. The objective of this sorting is to keep all

until they are returned to Windward. The examination will be performed on aluminum foil (re-applied between sampling locations), and Dr. Kohn will be wearing nitrile powder-free examination gloves to minimize contamination of the samples during the imposex analysis.

Helle Andersen will be present during the imposex analysis to track individual gastropods, to ensure that the vials with the females are labeled appropriately, and to make notations on the COC forms at Dr. Kohn's laboratory. After completion of the imposex analysis on June 21, 2004, all gastropod samples will be returned to Windward and stored in a sample storage freezer. The decision whether to archive these samples or send them to Columbia Analytical Services for chemical analyses will be made in consultation with EPA and Ecology.

8.0 Data Reporting

The results of the gastropod pilot survey will be presented in a technical memorandum submitted on July 7, 2004, prior to a meeting to be scheduled with EPA and Ecology in mid July. The memorandum will present, at a minimum, the sampling methods, survey locations, field notes, photos, and tables summarizing the estimated abundances and biomass of the collected gastropods and potential surrogate organisms.

9.0 References

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10.0 Oversize Figures

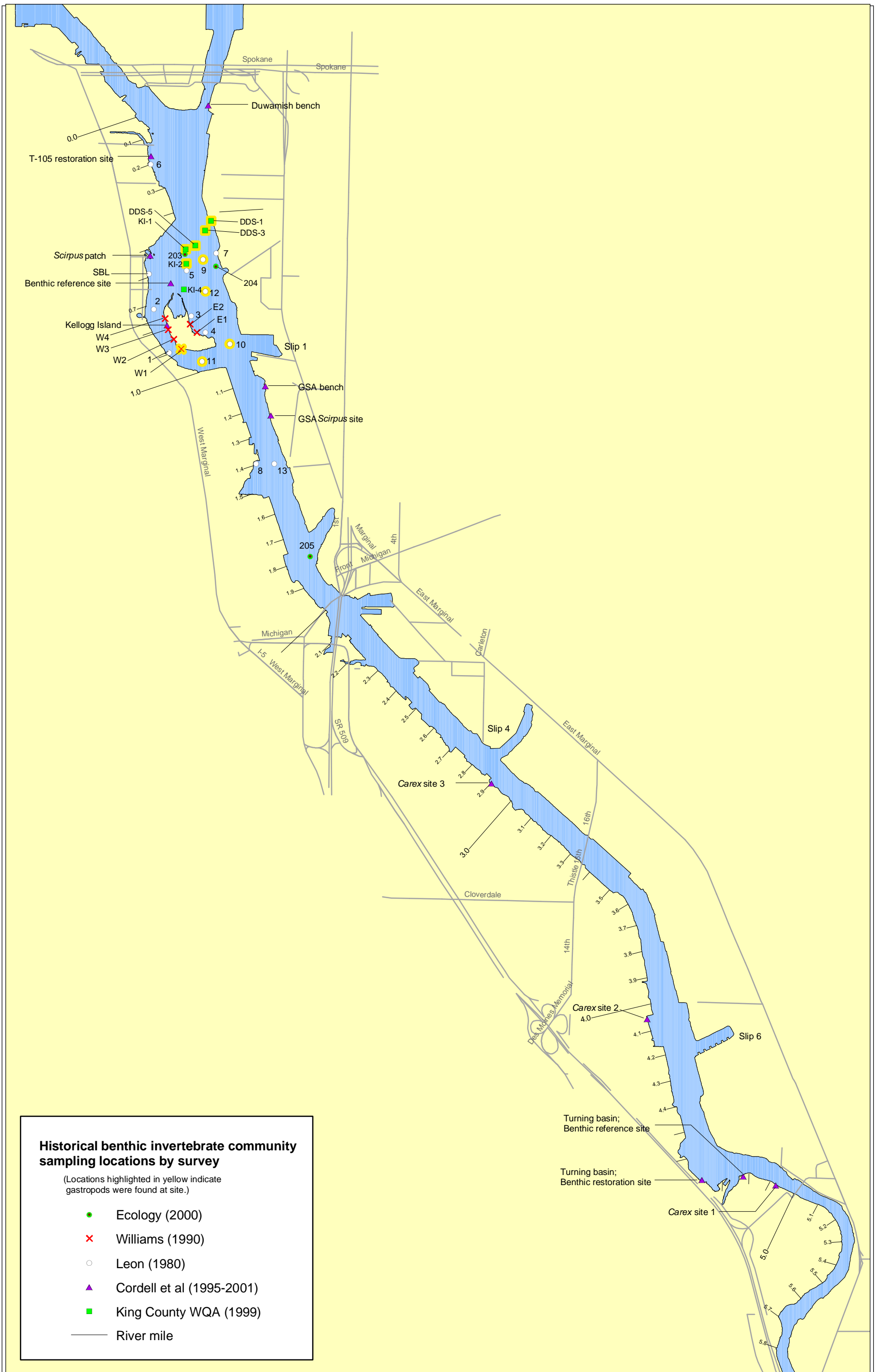
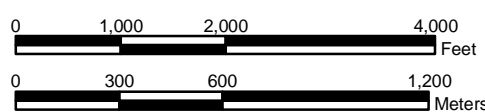


Figure 3-1. Historical benthic invertebrate community sampling locations in the Lower Duwamish Waterway.



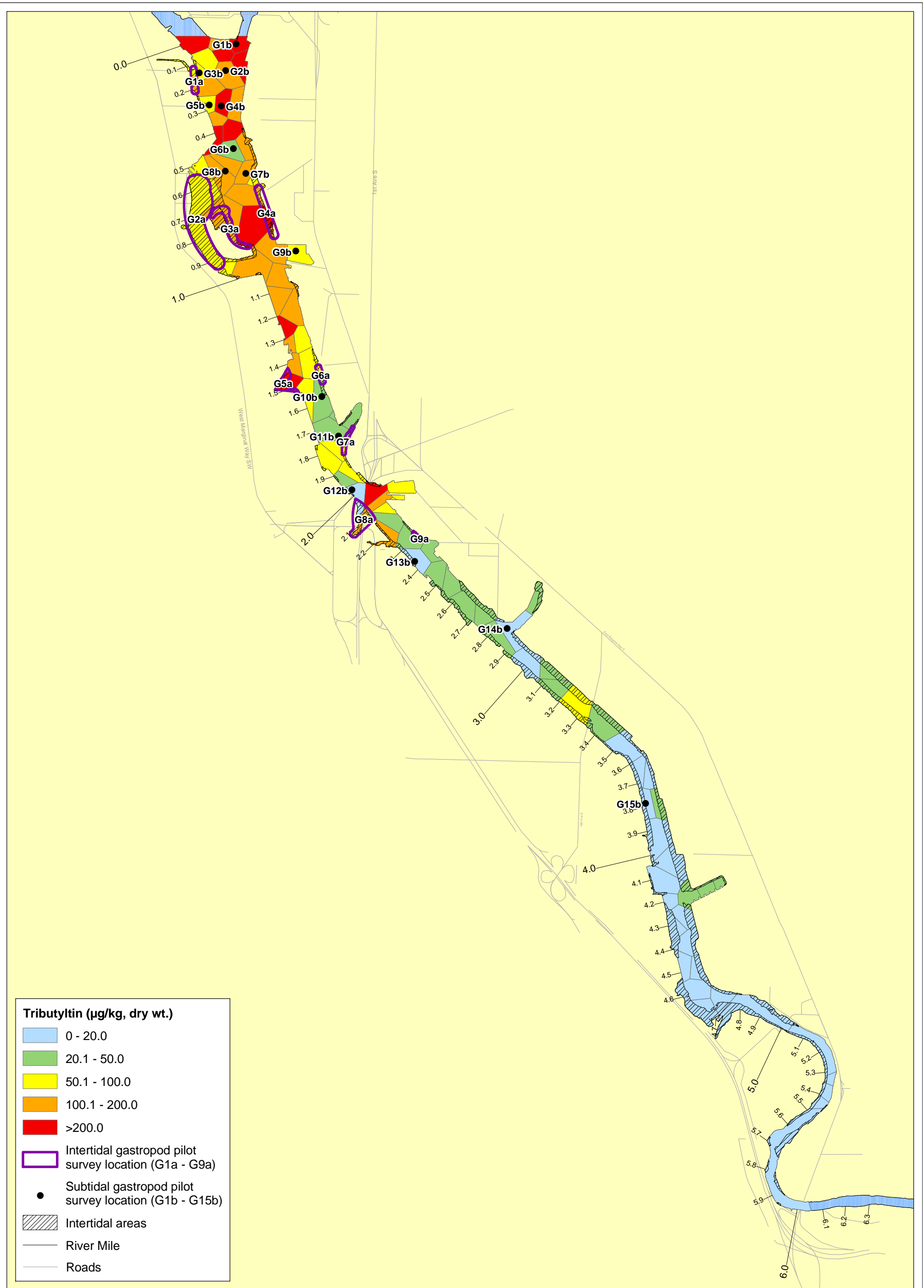


Figure 5-1. Gastropod pilot survey locations and tributyltin concentrations in LDW surface sediments (half DL) by thiesen polygon



Appendix A Forms
