Lower Duwamish Waterway Group

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

Lower Duwamish Waterway Remedial Investigation

TECHNICAL MEMORANDUM: 2005 GASTROPOD IMPOSEX STUDY 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 Imposex Study
Quality Assurance Project Plan

Windward Project Manager	Name	Date
Windward Field Coordinator	Name	Date
Windward QA/QC Manager	Name	Date
EPA Project Manager	Name	Date
Ecology Project Manager	Name	Date

Distribution List

This list identifies all individuals to receive a copy of the approved gastropod imposex study technical memorandum, either in hard copy or electronic format, as well as any subsequent revisions.

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Acronyms

ACRONYM	Definition
COC	chain of custody
COPC	chemical of potential concern
DGPS	differential global positioning system
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
PM	project manager
QA/QC	quality assurance/quality control
QAPP	Quality Assurance Project Plan
RI	Remedial Investigation
RM	river mile
TM	task manager
TBT	tributyltin
Windward	Windward Environmental LLC

1.0 Introduction

This technical memorandum describes the objectives, methods, and procedures for conducting a gastropod imposex study in the Lower Duwamish Waterway (LDW) in 2005. A pilot gastropod survey was conducted in 2004 to assess the abundance of gastropods in the LDW. The pilot study also evaluated the prevalence of imposex among LDW gastropods to determine the feasibility of evaluating the imposex endpoint for gastropods exposed *in situ* as part of the ecological risk assessment (ERA) (Windward 2004b). Based on findings from the 2004 pilot survey, the Lower Duwamish Waterway Group (LDWG), the Washington Department of Ecology (Ecology), and the US Environmental Protection Agency (EPA) determined that sufficient numbers and species of gastropods could be collected to directly assess risks to gastropods by assessing imposex in field-collected gastropods, and that an additional survey for imposex in gastropods collected from subtidal areas of the LDW should be conducted in 2005.

Data from this effort as well as the pilot study, will be used to support the Phase 2 ERA, as described in the Work Plan for the Phase 2 Remedial Investigation (RI) (Windward 2004a). Section 3.1.5 of the Work Plan identified the need for additional data to assess risks from tributyltin (TBT) to benthic invertebrates. Of the benthic invertebrates that may inhabit the LDW, meso- and neogastropods[1] have been identified as particularly sensitive to TBT (Meador et al. 2002). At sufficiently high tissue concentrations, TBT is known to cause the development of male sexual organs in females in some meso- and neogastropod species, a condition known as imposex (Gibbs et al. 1988). If sufficiently pronounced, imposex can interfere with gastropod reproduction and potentially result in population-level effects (Meador et al. 2002).

This technical 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 – imposex analysis
- Section 8 – data reporting
- Section 9 – references
- Section 10 – oversize figures

[1] Mesogastropods and neogastropods are snails in the taxonomic order of Mesogastropoda and Neogastropoda , respectively.

2.0 Project Organization

This section presents the overall project organization for the 2005 gastropod imposex study, 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 this technical memorandum, and interpretation of the results of the study. 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:

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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 the provisions of 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 field QA/QC oversight. Ms. Andersen will also be responsible for communicating with Dr. Alan Kohn and ensuring that the gastropod samples are delivered to his laboratory. Ms. Andersen can be reached as follows:

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

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3.0 Problem Definition/Background

The Phase 2 RI work plan (Windward 2004a) identified the need for additional data to assess risks from TBT to benthic invertebrates, in particular meso- and neogastropods. This section presents: 1) the results from two studies conducted by LDWG in 2004 that gathered information on the abundance and distribution of gastropods in the LDW, 2) the results from other studies that included analysis of TBT in LDW surface sediments, and 3) and the objectives for the 2005 gastropod imposex analysis described in this technical memorandum.

The first gastropod study conducted in 2004 was a pilot survey conducted at 9 intertidal and 15 subtidal locations between river miles (RMs)[2] 0.0 and 3.8 (Windward 2004b). The objectives of the pilot survey were to assess the presence of gastropods in intertidal and subtidal habitats of the LDW, and to determine whether sufficient numbers of gastropods were present to allow for their collection and chemical analysis in a subsequent sampling effort. A secondary objective was to determine whether imposex occurred in gastropods found in the LDW, and to determine the feasibility of assessing, as part of the LDW Phase 2 ERA, imposex among gastropods exposed to TBT *in situ*.

No gastropods were found at any of the nine intertidal locations. A total of 739 gastropods distributed over 11 taxa (species or genus) were found at the 15 subtidal locations at RMs 0.0 to 2.2.[3] Of the 11 taxa, five were in the order Mesogastropoda and three were in the order Neogastropoda. The most abundant species was the neogastropod *Astyris gausapata* (formerly *Mitrella goldii*), which accounted for 86% of all collected gastropods. This species was found at all subtidal locations up to RM 1.0. Further upstream, only two specimens of *A. gausapata* were collected. The next most common neogastropod, *Nassarius mendicus*, which accounted for 4% of all collected gastropods, was also primarily found in the area below RM 1.0. Very low numbers of mesogastropods and other neogastropods (< 10 individuals per species) were collected between RM 0.0 and RM 1.8, and no gastropods were found upstream of RM 2.2.

[2] River mile (RM) designations are referenced to the southern end of Harbor Island (RM 0).

[3] No gastropods were found in locations upstream of RM 2.2. TBT concentrations in upstream surface sediments were lower than the concentrations in surface sediments collected between RM 0.0-1.8.

A total of 38 individual gastropods were evaluated in the imposex analysis. Four *A. gausapata* males and ten females were evaluated. None of the female *A. gausapata* showed evidence of imposex. Twenty-four specimens of *N. mendicus* were also evaluated, of which 14 were male and 10 were female. All females examined had imposex at a stage that would not cause sterility (Windward 2004b).

The second study in 2004 included collection of benthic community samples at 12 intertidal and 14 subtidal locations (Windward 2005b). One of the objectives of this study was to collect additional data within representative LDW habitats on the general composition, relative abundances, and distribution of the diverse groups of invertebrates in the benthic community, and to provide this information in areas where benthic invertebrate tissue samples were collected for chemical analyses.

No gastropods were found at any of the 12 intertidal locations. A total of 319 gastropods distributed over 14 taxa were collected at the 14 subtidal locations. Of these 14 taxa, two were in the order Mesogastropoda and four were in the order Neogastropoda. The most abundant species was the mesogastropod *Alvania compacta,* which accounted for 77% of all collected gastropods. This species was found at most subtidal locations up to RM 2.2. The neogastropod *A. gausapata*, the next most common gastropod, accounting for 8% of all the collected gastropods, was found at subtidal locations up to RM 1.4.

The difference in species composition between the two surveys may have been a result of different sampling methods. During the first survey, samples were sorted in the field without magnification, whereas during the second survey samples, the samples were sorted in the laboratory using a dissecting microscope. The number of small gastropods (e.g., *A compacta*) counted in the second survey may have been greater than in the first survey because the use of a dissecting microscope made it possible to see very small gastropods missed in the field. Table 3-1 and Figure 3-1 summarize the meso- and neogastropods collected during the two sampling efforts in 2004.

Table 3-1. Meso- and neogastropods collected during the two benthic invertebrate sampling efforts in 2004

Location ID	River Mile	Mesogastropoda					Neogastropoda			
		Alvania compacta	*Cryptonatica affinis*	*Euspira* sp.	*Lacuna vincta*	*Trichotropis cancellata*	*Astyris gausapata*	*Kutzia arteaga*	*Nassarius mendicus*	*Olivella baetica*
G1b	0.0	2	0	0	0	0	69	0	7	0
G2b	0.1	0	0	1	0	1	222	0	8	0
G3b	0.1	0	0	0	0	0	42	1	1	0
B1b	0.1	8	0	0	1	0	0	3	1	1
G4b	0.3	0	0	0	0	0	73	3	1	0
G5b	0.3	0	0	0	0	1	2	0	1	0
G6b	0.5	0	0	0	0	0	66	1	5	0
G7b	0.6	0	0	0	1	0	3	0	1	0
G8b	0.6	3	0	0	1	0	140	0	2	0
BCA-4	0.6	123	0	0	0	0	14	0	0	0
B2b	0.8	9	0	0	0	0	6	1	0	0
G9b	0.9	0	0	3	0	0	14	0	0	0
B3b	1.0	28	0	0	0	0	0	0	0	0
B4b	1.4	58	0	0	0	0	4	0	0	0
B5b	1.5	0	0	0	0	0	0	0	0	0
BCA-5	1.5	3	0	0	0	0	0	0	0	0
G10b	1.6	0	0	2	0	0	1	0	1	0
BCA-2	1.7	13	0	0	0	0	0	0	0	0
G11b	1.8	0	1	0	0	0	0	0	0	0
G12b	2.0	0	0	0	0	0	1	0	0	0
B6b	2.2	2	0	0	0	0	0	0	0	0
G13b	2.4	0	0	0	0	0	0	0	0	0
B7b	2.7	0	0	0	0	0	0	0	0	0
G14b	2.8	0	0	0	0	0	0	0	0	0
G15b	3.8	0	0	0	0	0	0	0	0	0
B9b	3.9	0	0	0	0	0	0	0	0	0
B8b	4.2	0	0	0	0	0	0	0	0	0
B10b	4.3	0	0	0	0	0	0	0	0	0
BCA-6	4.6	0	0	0	0	0	0	0	0	0
TOTAL		**249**	**1**	**6**	**3**	**2**	**657**	**9**	**28**	**1**

In addition to the studies discussed above, additional data on the distribution and concentrations of TBT in LDW surface sediments were collected during two studies in August-September 2004 and January-March 2005. In conjunction with the collection of benthic invertebrate tissue samples in summer 2004, co-located surface sediment composite samples were collected for chemical analyses at ten intertidal and ten subtidal locations, for a total of 20 sediment samples (Windward 2005b). In addition, clam tissue samples and co-located surface sediment composite samples were collected at ten intertidal locations, with two sediment samples collected at each of four of those locations, for a total of 14 sediment samples. All 34 surface sediment samples were analyzed for TBT. These sediment data were presented in the benthic invertebrate chemistry data report (Windward 2005a).

In 2005, surface sediment grab samples were collected to assess the areal extent, concentration, and distribution of LDW chemicals of potential concern (COPCs), including TBT, for which relatively few samples were available in Phase 1 (Windward 2005c). TBT analyses were conducted on 37 surface sediment samples collected in 2005 in the LDW. The TBT data will be presented along with all of the other sediment chemistry data in the Round 1 and 2 surface sediment data reports, to be submitted later in 2005. Table 3-2 summarizes TBT concentrations in surface sediment samples collected in 2004 and 2005, and Figure 3-2 presents these TBT concentrations along with historical TBT data from Phase 1.

Table 3-2. Tributyltin concentrations in surface sediment samples collected in 2004 and 2005

Sample ID [a]	River Mile	TBT (as ion) sediment concentration (µg/kg dw)	Sample ID	River Mile	TBT (as ion) sediment concentration (µg/kg dw)
LDW-SS2[b]	0.0	10	LDW-SS53	1.4	6.3
LDW-SS3[b]	0.0	9.4	LDW-SS55	1.4	16
LDW-SS4[b]	0.0	19	LDW-SS56	1.4	96
LDW-SS6	0.0	20	LDW-C4	1.4	3.4
LWD-SS7	0.1	7.5	LDW-B4a	1.5	32
LDW-SS8	0.1	20	LDW-B5b	1.5	30
LDW-B1b	0.1	2,300 J	LDW-SS58	1.5	28
LDW-B1a	0.2	0.35 J	LDW-SS67	1.8	3.7 U
LDW-C1	0.2	0.28 J	LDW-C5	1.8	6.5
LDW-SS14	0.3	3.7 U	LDW-SS74	2.0	110
LDW-SS15	0.3	28	LDW-C6	2.1	1.8
LDW-SS16	0.3	5.5	LDW-B6a	2.1	2.3
LDW-SS20	0.4	12	LDW-SS78	2.1	19
LDW-C3-S1	0.6	0.74 J	LDW-SS79	2.1	3.7 U
LDW-C3-S2	0.6	0.39 J	LDW-B6b	2.2	20
LDW-C2-S1	0.7	4.3	LDW-B5a-2	2.3	6.4

Sample ID [a]	River Mile	TBT (as ion) sediment concentration (µg/kg dw)		Sample ID	River Mile	TBT (as ion) sediment concentration (µg/kg dw)
LDW-SS28	0.7	7.5		LDW-B7b	2.7	13
LDW-B3a	0.7	2.1		LDW-C9	2.8	1.3 J
LDW-SS27	0.8	28		LDW-C7-S1	2.9	2.0
LWD-B2b	0.8	63		LDW-C7-S2	2.9	1.1 J
LDW-C2-S2	0.9	1.2 J		LDW-C8	2.9	7.0
LDW-SS33	0.9	3.9 U		LDW-B7a	3.1	5.6
LDW-SS34	0.9	5.4		LDW-SS107	3.4	3.8 U
LDW-B2a	0.9	22		LDW-SS108	3.5	8.7
LDW-SS31[c]	1.0	81		LDW-B8a	3.5	5.8
LDW-B3b[c]	1.0	320		LDW-C10-S1	3.6	3.8
LDW-SS32	1.0	33		LDW-C10-S2	3.6	2.7
LDW-SS38	1.1	23		LDW-B9b	3.9	6.7
LDW-SS41	1.2	18 J		LDW-SS124	4.0	9.8
LDW-SS43	1.2	99		LDW-SS131	4.2	53
LDW-SS45	1.3	260		LDW-B8b	4.2	1.7 J
LDW-SS46	1.3	3,000		LDW-SS133	4.3	3.7 U
LDW-SS47	1.3	230 J		LDW-B10b	4.3	2.3
LDW-B4b	1.4	96		LDW-B9a	4.5	1.6 J
LDW-SS49	1.4	140		LDW-B10a	4.7	3.6
LDW-SS51	1.4	28				

Data qualifiers: U - not detected at reporting limit shown; J - estimated concentration

[a] Samples containing the character B and C are from the 2004 benthic invertebrate sampling event (Windward 2005b); samples with the characters SS are from the 2005 surface sediment sampling event (Windward 2005c)

[b] Historical samples were collected in the area from RM 1.3 to 1.6 that are referred to a SS1 through SS5 on Figure 3-2. These locations and samples are different than Phase 2 samples SS2 through SS4 in this table, and thus have different TBT concentrations.

[c] Samples were collected within approximately 4 m of each other during different sampling events using different compositing techniques

The results of the gastropod pilot survey showed that it is possible to directly assess risks to gastropods by assessing imposex in field-collected gastropods. The objectives of the 2005 gastropod imposex study, described in this technical memorandum, are:

- to examine additional specimens of the neogastropod species assessed in 2004 for imposex
- to sample additional locations where relatively high TBT concentrations have been measured in surface sediments
- to perform imposex analysis on mesogastropods and other neogastropods to the extent they are available

The study will provide additional data on the extent of imposex in meso- and neogastropods collected in the LDW to complete the assessment of risk from TBT to gastropods, the most sensitive benthic invertebrate group to TBT[4]. The study design incorporates Phase 2 data on the distribution of TBT in LDW surface sediment with the goal of assessing imposex directly in additional representatives of meso- and neogastropods.

4.0 Project/Task Description and Schedule

To address the data needs and study objectives identified in Section 3.0, a field sampling effort will be conducted August 8 – 12, 2005 to collect gastropods for imposex analysis. The imposex analysis will be conducted August 9 – 19, 2005 by Dr. Alan Kohn at the University of Washington, who will submit a summary report of the imposex analysis to Windward. A draft report presenting the results of the imposex analysis will be submitted to EPA and Ecology on November 4, 2005.

5.0 Data Generation and Acquisition

This section describes the sampling design and methods that will be used to conduct the 2005 gastropod imposex study. In addition, this section presents the sample identification scheme and a list of the field equipment needed to collect gastropods.

5.1 SAMPLING DESIGN

This study will focus on the subtidal area in the lower 1.5 miles (i.e., RM 0.0-1.5) of the LDW. The 2004 sampling efforts (Windward 2005a, b) showed that gastropods were present in this area in sufficient abundance to evaluate imposex in this area. As shown in Table 3-1, three meso- or neogastropod species were relatively abundant (> 20 individuals) in 2004; six other meso- or neogastropod species were present but less abundant (< 10 individuals each or 22 individuals in total).

Subtidal sampling locations for the gastropod imposex study were selected based on the following objectives:

- to optimize the likelihood of collecting several different species of meso- and neogastropods
- to collect gastropods from additional areas that exhibited relatively high TBT concentrations in surface sediment during the 2004/2005 sampling efforts

Six subtidal sampling areas will be located between RM 0.0 and RM 1.5 (Table 5-1 and Figure 3-2). The sample identification scheme will be the same as that used for the gastropod pilot survey (see Section 5.3). Fifteen subtidal locations were sampled

[4] Risk from TBT to other benthic invertebrates will be evaluated using a tissue-based approach in the Phase 2 ERA.

in the pilot survey; the current survey will start with location number 16. To increase the likelihood of collecting several different species, 10 tows with the benthic sledge will be performed at each location and two of the selected locations either will reoccupy (G16b and G18b) or will be located near (G17b, G19b, and G20b) sampling locations where multiple gastropod species were found in 2004.

Table 5-1. Sampling locations and rationale for the 2005 gastropod imposex study

Location ID for This Study	Previous Location ID(s)	Sediment Concentration of TBT (as ion) (µg/kg dw) [a]		Rationale
		At Target Location	At Nearby Locations (Location ID, Distance)	
LDW-G16b	LDW-B1b	2,300 J	96 (K06, 16 m)	Second highest TBT concentration in surface sediments in 2004/2005; highest number of species (5) of meso- or neogastropods collected in 2004; 15 individual gastropods collected
LDW-G17b	DR002	320	7.7(LDW-SS7, 60 m)	High historical TBT surface sediment concentration based on Phase 1 data; no gastropods have been collected at this location but three species of meso- and neogastropods were collected at nearby locations (LDW-G1b and LDW-G2b)
LDW-G18b	LDW-G4b (K04)	350	28 (LDW-SS15, 27 m)	Three species (77 individuals) of meso- or neogastropods collected at LDW-G4b; high historical TBT concentration in surface sediments based on Phase 1 data
LDW-G19b	DR072	250	63 (LDW-B2b, 124 m)	Three species (16 individuals) of meso- or neogastropods collected at LDW-B2b (124 m upstream); high historical TBT concentration in surface sediments based on Phase 1 data
LDW-G20b	DR021	170	100 (DR018, 108 m)	Two species (17 individuals) of meso- or neogastropods collected at LDW-G9b (115 m farther into Slip 1); high historical TBT concentration in surface sediments based on Phase 1 data
LDW-G21b	LDW-SS46	3,000	230 (LDW-SS47, 22 m) 258 (LDW-SS45, 42 m)	Highest TBT concentration in surface sediments in Phase 2; no gastropod information is available for this area, but 62 individual meso- or neogastropods were collected at LDW-B4b, located approximately 170 m to the southeast

[a] Sediment samples from LDW-G1b through LDW-G15b locations were not chemically analyzed in the 2004 pilot survey. Concentrations of TBT in sediment from Phase 1 locations are given instead.

The selected sampling locations include areas within the LDW that have historically had the highest TBT concentrations (Figure 5-1), representing worst-case exposure. The highest surface sediment concentrations of TBT are targeted by reoccupying locations B1b (2,300 µg/kg dw) and SS46 (3,000 µg/kg dw) (see Figure 3-2). Location G16b (near location B1b) also had the highest number of meso- and neogastropod species (5) in the 2004 benthic invertebrate study (Windward 2004c). At location

G20b in Slip 1, the sampling will be initiated at the mouth and the benthic sledge will be towed toward the middle of the slip. It will not be possible to sample at the head of the slip near location B3b because of debris on the bottom, including pilings, rocks, and cables. In addition, there is very limited space in this area for maneuvering a boat towing sampling gear. Location G21b (near location SS46) is located in an area along the west bank between RM 1.3 and 1.4 where there were relatively high TBT sediment concentrations (130-3,000 µg/kg dw) in 2005. No gastropod information is available for this area. However, two gastropod species, *A. gausapata* and *A. compacta*, were collected in relatively high numbers across the waterway at B4b, approximately 150 m away, indicating that it may be possible to collect meso- and neogastropods in this reach of the LDW. If no meso- or neogastropods are collected at location G21b or barges limit access at this location, the sampling effort will be expanded to cover the general area between SS45, SS46, and SS47 (Figure 3-2), where TBT concentrations ranged from 230 to 3,000 µg/kg dw (Table 3-2). If no meso- or neogastropods are collected in the general area between SS45, SS46, and SS47, the sampling effort will be moved to location B4b.

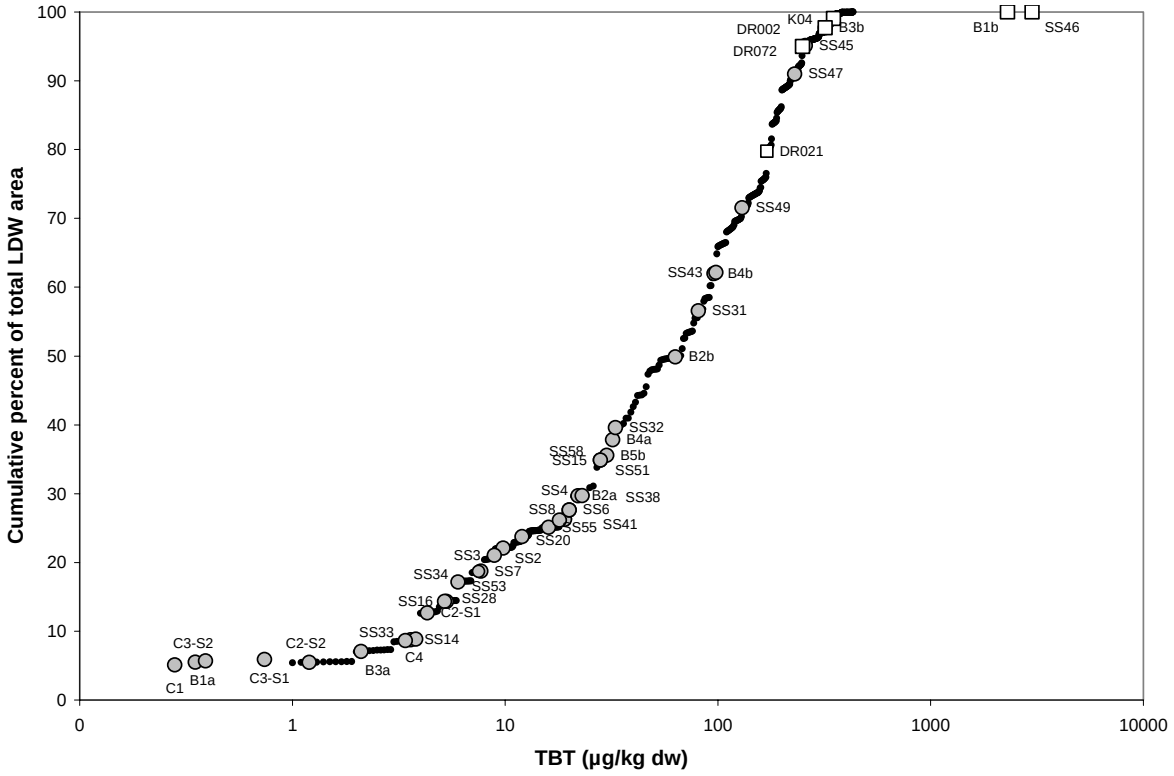


Figure 5-1 Cumulative frequency distribution of surface sediment TBT concentrations in the LDW

Note: This plot incorporates Phase 1 surface sediment TBT concentrations (black dots), Phase 2 surface sediment TBT concentrations at locations between RM 0.0 and 1.5 (gray circles), and surface sediment TBT concentrations at locations selected for this gastropod study (white squares)

The other locations, G17b-G20b, are also located in areas likely to have relatively high TBT concentrations (170 – 350 µg/kg dw) based on Phase 1 data. Coordinates for the six sampling locations are shown in Table 5-2.

Table 5-2. Target coordinates of 2005 gastropod imposex study locations

Location ID	Previous Location ID	Easting (x) [a]	Northing (y) [a]	Latitude	Longitude
LDW-G16b	LDW-B1b	1266302	210812	47 34.070	122 20.933
LDW-G17b	(DR002)	1267001	210863	47 34.080	122 20.760
LDW-G18b	LDW-G4b (K04)	1266551	209883	47 33.918	122 20.868
LDW-G19b	DR072	1267143	207371	47 33.507	122 20.712
LDW-G20b	DR021	1267822	206718	47 33.401	122 20.520
LDW-G21b	LDW-SS46	1267940	204779	47 33.083	122 20.506

[a] Both geographic and state plane coordinates (WA State Plane N, US feet) based on NAD 83 horizontal datum

5.2 Sampling Methods

5.2.1 Sample collection methods

Field sampling for the gastropod imposex study will be conducted over five days. One to two locations will be sampled each day, starting with the locations farthest downstream to increase the likelihood of collecting a sufficient numbers of individuals and species of meso- and neogastropods. Sampling will be conducted with a benthic sledge. The benthic sledge consists of a metal frame 21 cm high by 60 cm wide (Figure 5-2). A bag (approximately 50 cm × 60 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 150 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 1:4 (1 m depth to 4 m of rope let out) and the duration of the tow will be 0.5 to 1 minute depending on the fullness of the bag. The scope and tow duration are based on the gastropod pilot survey (Windward 2004b).

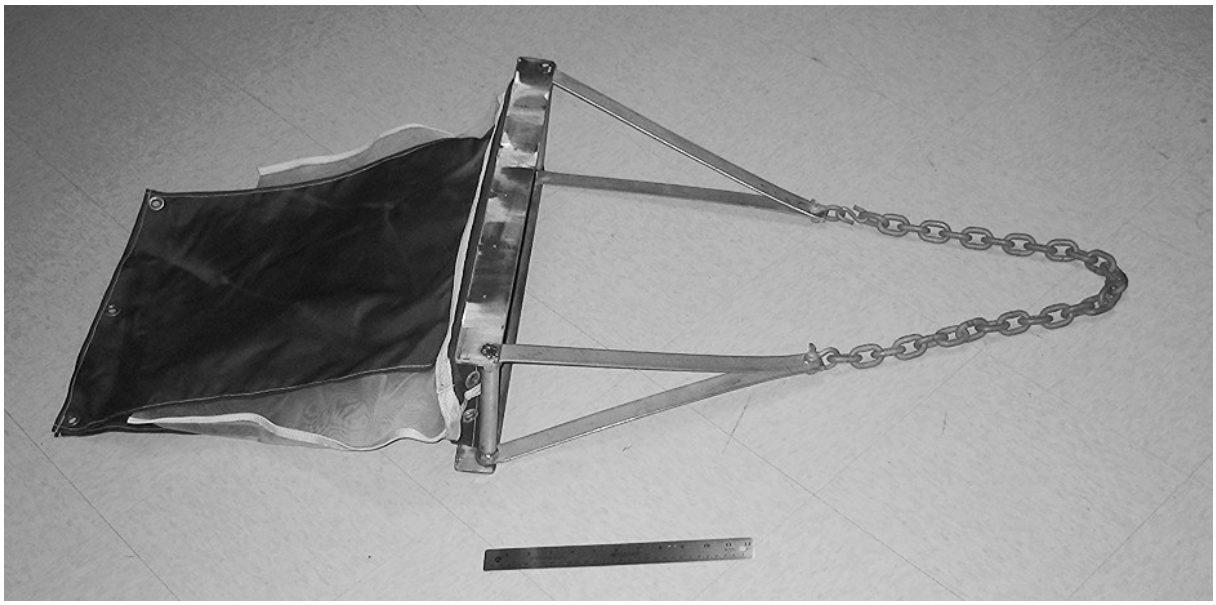


Figure 5-2. Benthic sledge sampler

At the completion of each designated tow, the sledge will slowly be winched out of the water and into the boat. The contents of the bag will be rinsed into a 1.0-mm mesh sieve, and the gastropods in the sieve will be collected and sorted into separate wide-mouth glass jars quarter-filled with Elliott Bay[5] surface water according to the following field identifications:[6] *A. gausapata, A. compacta*, *N. mendicus,* and all other gastropods. Ten tows will be performed within the general vicinity (<10-50 m) of each target location. Up to 100 individual of each of the three species (*A. gausapata, A. compacta*, *N. mendicus)* will be collected at each location and delivered to Dr. Kohn for imposex analysis.[7] If more than 100 individuals of these three species are present at the location, excess individuals will be returned to the sampling location. All other gastropod specimens collected in the ten tows will also be delivered to Dr. Kohn for imposex analysis. The number of gastropods per tow will be noted on the field collection form (see Appendix A). At the end of the sampling effort at each location, the number of gastropod jars will be noted in the field notebook, and the jars will be placed on ice in a cooler.

5.2.2 Navigation and station location methods

A global positioning system (GPS) will be used to identify the start of each sledge deployment. Sampling locations will be located using a Trimble NT300D differential

[5] 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.

[6] Identification of these gastropods in the field will be made by Helle Andersen; Dr Kohn will confirm the identification in the laboratory.

[7] Dr. Kohn will select from the largest individuals of these species at these locations to assess imposex. He will not have sufficient time to evaluate all individuals if such a large number of snails is collected (see Section 7.0)

GPS (DGPS). The DGPS includes a GPS receiver unit onboard the sampling vessel and a Coast Guard beacon differential receiver. The GPS unit will receive radio broadcasts of GPS signals from satellites. The Coast Guard beacon receiver will acquire corrections to the GPS signals to produce positioning accuracy to within 1-2 m. Northing and easting coordinates of the vessel will be updated every second and displayed directly on a computer onboard the vessel. The coordinates will then be processed in real time and stored at the time of sampling using the positioning data management software package. Washington State Plane Coordinates, North (NAD 83) will be used for the horizontal datum. Depth measurements will be provided by the boat's depth finder. Vertical elevations will be estimated by noting the tide level at the time of sampling.

5.3 SAMPLE IDENTIFICATION SCHEME

The sample identification scheme will be the same as that used for the gastropod pilot survey. The first three letters of the location identification number are "LDW" to identify the Lower Duwamish Waterway project area. The location naming convention uses sequential numbers prefaced by a "G" to indicate the gastropod study. Fifteen subtidal locations were sampled in the pilot survey so the current survey will start with number 16. As in the gastropod pilot survey, the subtidal locations will be identified with a "b" after the location number. The locations are numbered G16b (northernmost) to G21b (southernmost).The gastropod sample collected at location G17b would thus be identified as LDW-G17b.

5.4 FIELD EQUIPMENT

Field equipment needs for the study are listed in Table 5-3. This list will be consulted to ensure that all equipment is available in the field. As part of the mobilization process, each item will be double checked by the FC.

Table 5-3. Field equipment needs for the gastropod pilot survey

benthic sledge	digital camera	field notebooks
coolers	batteries	pens/pencils/Sharpies
wet ice	rubber work gloves	tide tables
spray bottle	first aid kit	technical memorandum
wide-mouth glass jars	duct tape	paper towels
forceps	personal flotation devices	field sample sheets
study area maps	chain-of-custody forms	labels
GPS	counters	

6.0 Sample Handling and Custody Requirements

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 location will be placed in appropriate-sized, wide-mouth glass jars containing Elliott Bay 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.

The samples will be kept on ice in the field and during transport to Dr. Alan Kohn. The samples will be hand-delivered to Dr. Kohn on the same day as they are collected.

6.2 SAMPLE CUSTODY PROCEDURES

Custody procedures will be used during collection, transport, and storage of the gastropod samples. 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 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 delivered to Dr. Kohn

The FC will be responsible for all sample 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 delivery, 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 be signed at delivery. Copies of all COC forms will be retained and included as an appendix to the results report.

7.0 Imposex Analysis

Imposex analyses will be performed by Dr. Alan Kohn at the University of Washington. In the 2004 gastropod pilot survey, Dr. Kohn requested to receive only meso- and neogastropods with a shell height greater than 1 cm to ensure that he would be able to perform the imposex analysis (Windward 2004b). For the 2005 imposex study, there will be no shell height requirement[8] and Dr. Kohn will receive up to 100 gastropods of each species collected at each location. Dr. Kohn will evaluate as many meso- and neogastropods as possible, during the 40 hours he has allocated to this project, focusing on larger meso- and neogastropods on which he can perform the imposex analysis. Upon receipt of the gastropods, he will conduct an initial screen to identify any species, in addition to *Nassarius mendicus* identified with Stage 2 imposex last year, showing any evidence of imposex. Based on this initial assessment and the diversity and number gastropods available, Dr. Kohn will assess as many individuals of meso- and neogastropod species showing signs of imposex as possible within the allocated time period, while also assessing as many different species of meso- and neogastropods as possible at each location.

Table 7-1 presents the mean shell heights measured during the 2004 gastropod-related surveys and maximum shell heights reported in the literature. Note that the relatively common *Alvania compacta* may be difficult to see without magnification because of its small size, and could therefore potentially be missed during collection of gastropods in the field. In addition, it may be difficult to perform imposex analysis on *A. compacta* because of its small size.

Table 7-1. Shell height of meso- and neogastropods collected during the two field sampling efforts in 2004 and as reported in the literature

Order	Species/Genus	Mean Shell Height (cm ± SD) [a]	Maximum Shell Height (cm) [b]
Mesogastropoda	*Alvania compacta*	0.22 ± 0.03	0.3
	Cryptonatica affinis	0.51	3.7
	Euspira sp.	0.28 ± 0.10	0.8-4.2[c]
	Lacuna vincta	0.70 ± 0.01	1.3
	Trichotropis cancellata	0.61 ± 0.36	2.5

[8] There is no shell height requirement in the 2005 survey because Dr. Kohn believes he may be able to assess imposex in gastropods with shell heights smaller than 1 cm based on his experience in 2004.

ORDER	SPECIES/GENUS	MEAN SHELL HEIGHT (cm ± SD) [a]	MAXIMUM SHELL HEIGHT (cm) [b]
Neogastropoda	*Astyris gausapata*	0.78 ± 0.19	2.5
	Kurtzia arteaga	0.87 ± 0.20	1.0
	Nassarius mendicus	1.20 ± 0.14	2.5
	Olivella baetica [d]	nm	1.5

[a] Mean of gastropod shell heights measured during the 2004 surveys

[b] Maximum shell height is based on a search of the literature

[c] Great interspecies variation in size reported in the literature

[d] Collected during the taxonomic identification of benthic invertebrate communities, and therefore, not measured for shell height

SD – standard deviation

nm – not measured

The imposex analysis will be initiated by Dr. Kohn identifying each gastropod to species or genus. Then the shells will be cracked carefully with a small hammer so as not to kill the gastropod, and the gender of each gastropod will be determined (imposex can most easily be determined on live gastropods). The males will be returned to the sampling jar and stored in coolers with ice. The females will be examined for imposex using the approaches reported in Oehlmann et al. (1991), Spence et al. (1990), and Gibbs et al. (1988). Oehlmann et al. (1991) determined the imposex stage based on the presence of male reproductive organs in females, including vas deferens (a sperm-carrying duct) and penis (Table 7-2). If the vas deferens cannot be seen, as occurred during the 2004 imposex analysis (Windward 2004b), the level of imposex will be assessed by measuring the penis length of both males and females and using the relative penis size (RPS) approach (Gibbs et al. 1988). The RPS index will be calculated using the following equation:

$$\text{RPS index} = \frac{\text{mean length of female penis}^3}{\text{mean length of male penis}^3} \times 100 \qquad \textbf{Equation 1}$$

The imposex evaluation of each gastropod will be noted on the laboratory imposex form (see Appendix A). After examination, female gastropods will be placed in separate vials and stored on ice for further reference until completion of the imposex analysis. When the evaluation is complete, all gastropods will be euthanized (frozen) and discarded.

Table 7-2. Characteristics of imposex stages (Oehlmann et al. 1991)

IMPOSEX STAGE	CHARACTERISTICS
1	Development of small penis or small section of vas deferens
2	Development of either: a. larger penis with a penis duct, or b. two sections of vas deferens, or c. both a penis and a vas deferens section
3	Development of either: a. larger penis with vas deferens section, or b. a complete vas deferens, or c. a larger penis with a penis duct and a vas deferens section
4	Development of a larger penis with penis duct and a complete vas deferens (last fertile imposex stage)
5	Development of a prostate gland or occlusion of the vulva (infertile stage)
6	Infertile stage with aborted capsules

8.0 Data Reporting

The results of the gastropod imposex study will be presented in a report that will be submitted to EPA and Ecology in draft on November 4, 2005. The report will present the sampling locations and methods, field notes, photos, enumeration of meso- and neogastropods to genus or species, and results of the imposex analysis.

9.0 References

Gibbs PE, Pascoe PL, Burt GR. 1988. Sex change in the female dog-whelk, *Nucella lapidis*, induced by tributyltin from antifouling paints. J Mar Biol Ass UK 68:715-731.

Meador JP, Collier TK, Stein JE. 2002. Determination of a tissue and sediment threshold for tributyltin to protect prey species of juvenile salmonids listed under the US Endangered Species Act. Aquat Conserv: Mar Freshw Ecosys 12:539-551.

Oehlmann J, Stroben E, Fioroni P. 1991. The morphological expression of imposex in *Nucella lapillus* (Linnaeus) (Gastropoda: Muricidae). J Moll Stud 57:375-390.

Spence SK, Bryan GW, Gibbs PE, Masters D, Morris L, Hawkins SJ. 1990. Effects of TBT contamination on *Nucella* populations. Func Ecol 4:425-432.

Windward. 2004a. Lower Duwamish Waterway remedial investigation. Task 8: Phase 2 RI work plan. Prepared for Lower Duwamish Waterway Group. Windward Environmental LLC, Seattle, WA.

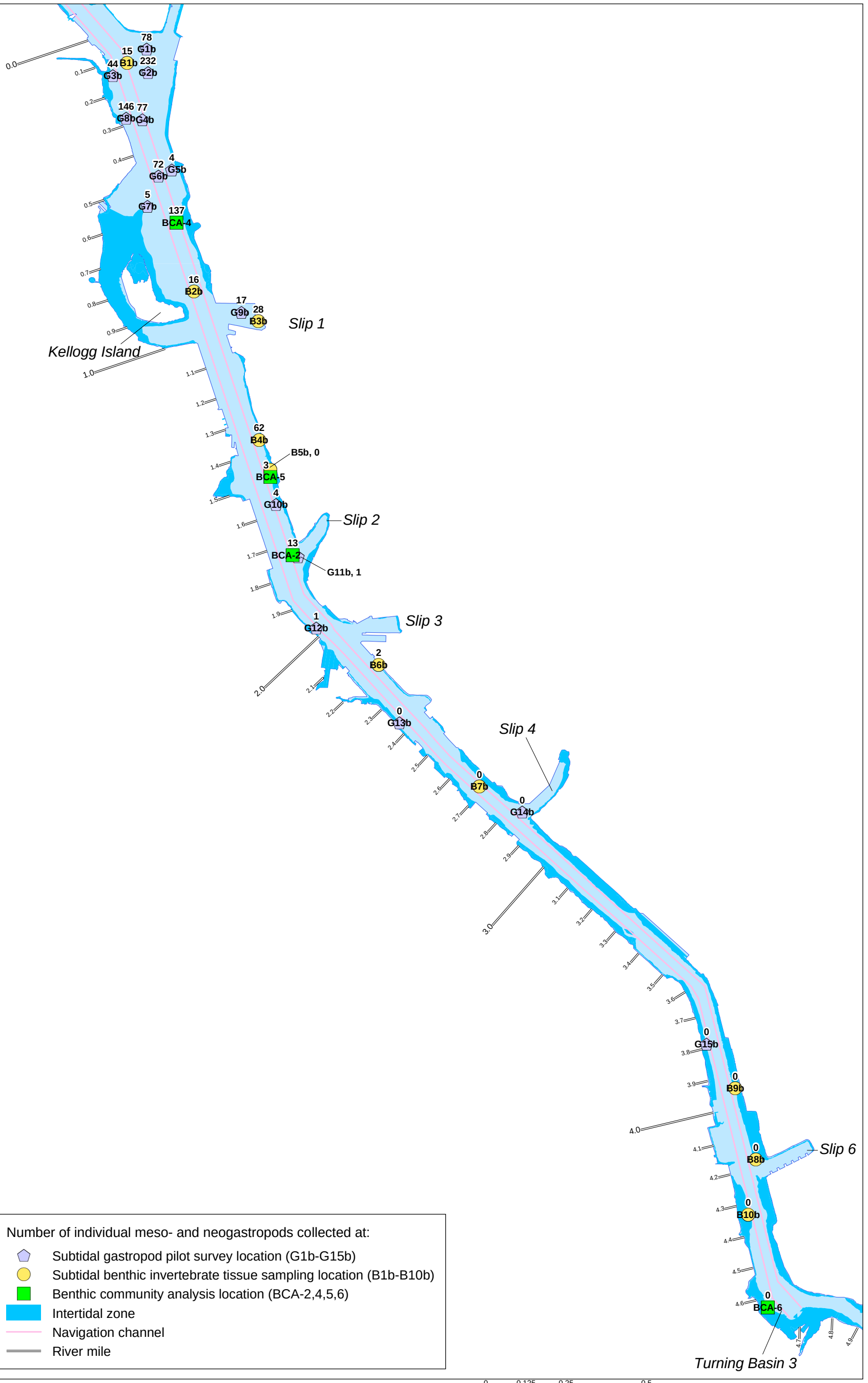
Windward. 2004b. Lower Duwamish Waterway remedial investigation. Technical memorandum: Gastropod pilot survey results. Windward Environmental LLC, Seattle, WA.

Windward. 2005a. Lower Duwamish Waterway remedial investigation. Data report: Chemical analyses of benthic invertebrate and clam tissue samples and co-located sediment samples. Prepared for Lower Duwamish Waterway Group. Windward Environmental LLC, Seattle, WA.

Windward. 2005b. Lower Duwamish Waterway remedial investigation. Data report: Taxonomic identifications of benthic invertebrate communities. Prepared for Lower Duwamish Waterway Group. Windward Environmental LLC, Seattle, WA.

Windward. 2005c. Lower Duwamish Waterway remedial investigation. Quality assurance project plan: surface sediment sampling and toxicity testing of the Lower Duwamish Waterway. Prepared for Lower Duwamish Waterway Group. Windward Environmental LLC, Seattle, WA.

10.0 Oversize Figures



Number of individual meso- and neogastropods collected at:

- Subtidal gastropod pilot survey location (G1b-G15b)
- Subtidal benthic invertebrate tissue sampling location (B1b-B10b)
- Benthic community analysis location (BCA-2,4,5,6)
- Intertidal zone
- Navigation channel
- River mile

0 0.125 0.25 0.5 Miles

0 0.125 0.25 0.5 Kilometers

N

Wind Ward environmental LLC

Figure 3-1. Total number of individual meso- and neo-gastropods collected at LDW sampling locations in 2004

Prepared by STS 02/28/05, updated 04/15/05, 05/07/05. 06/14/05 Map 1730

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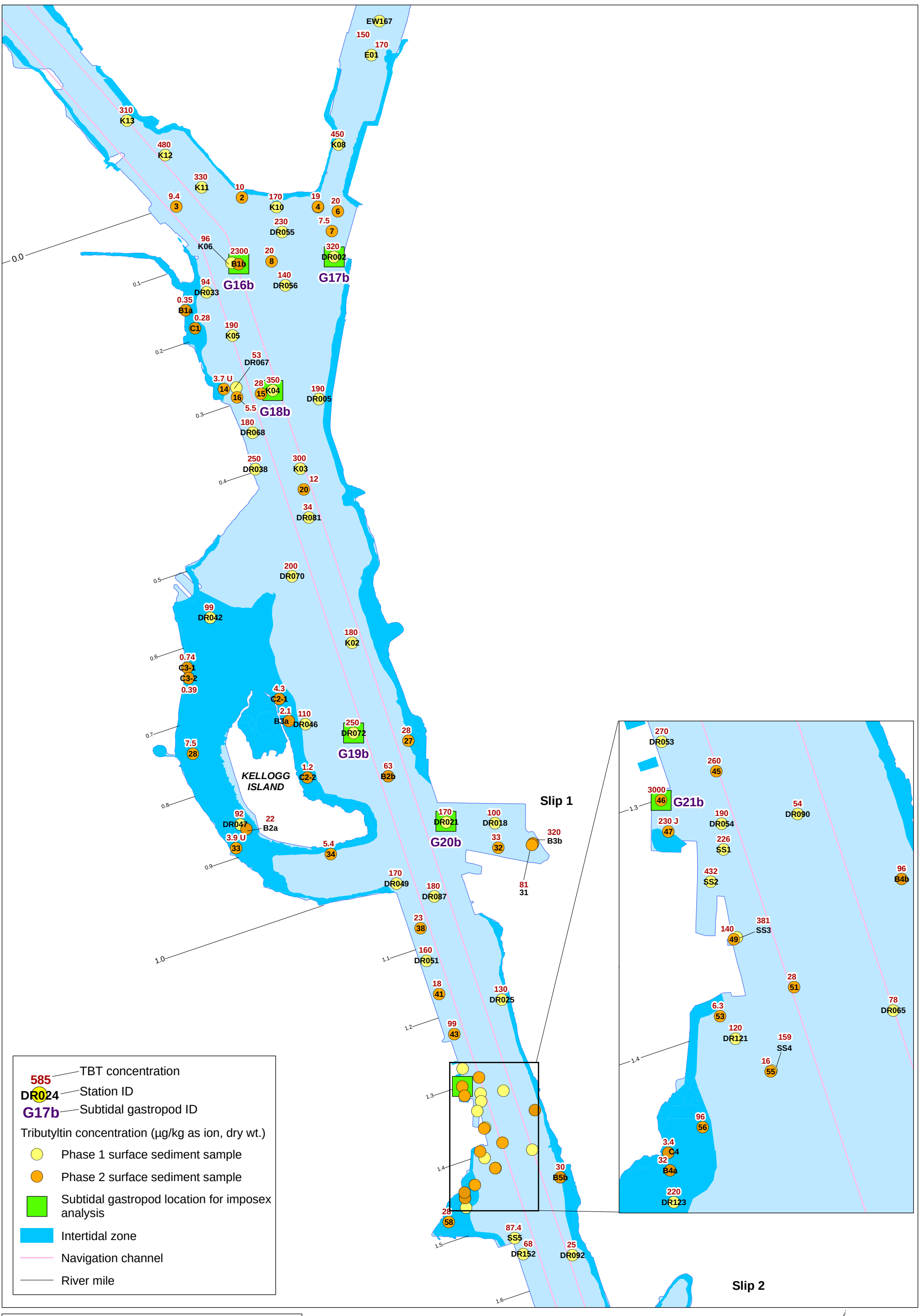

Figure 3-2. Phase 1 and 2 TBT concentrations in surface sediment samples and gastropod imposex sampling locations (RM 0.0-1.6)

Prepared by STS 05/03/05, updated 07/29/05, 08/01/05, 08/09/05 Map 1814

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Appendix A Gastropod Forms

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GASTROPOD FIELD COLLECTION FORM

Project Name: _____ **Project no.** _____

Date: _____ **Location:** **X:**

Start/Stop time: _____ **Y:** _____

Sampling Method: _____ **Sample ID:** _____

Weather: _____ **Crew:** _____

Tow #	Coordinates	Number of Gastropods		Comments
1	**X:**	*N. mendicus:*		
	Y:	*A. gausapata:*		
		A. compacta:		
		Others:		
2	**X:**	*N. mendicus:*		
	Y:	*A. gausapata:*		
		A. compacta:		
		Others:		
3	**X:**	*N. mendicus:*		
	Y:	*A. gausapata:*		
		A. compacta:		
		Others:		
4	**X:**	*N. mendicus:*		
	Y:	*A. gausapata:*		
		A. compacta:		
		Others:		
5	**X:**	*N. mendicus:*		
	Y:	*A. gausapata:*		
		A. compacta:		
		Others:		
6	**X:**	*N. mendicus:*		
	Y:	*A. gausapata:*		
		A. compacta:		
		Others:		
7	**X:**	*N. mendicus:*		
	Y:	*A. gausapata:*		
		A. compacta:		
		Others:		
8	**X:**	*N. mendicus:*		
	Y:	*A. gausapata:*		
		A. compacta:		
		Others:		
9	**X:**	*N. mendicus:*		
	Y:	*A. gausapata:*		
		A. compacta:		
		Others:		
10	**X:**	*N. mendicus:*		
	Y:	*A. gausapata:*		
		A. compacta:		
		Others:		

GASTROPOD IMPOSEX LABORATORY FORM

Project Name: Lower Duwamish Waterway RI- Benthic Invertebrates **Project no.** 04-08-06-21

Date: _____ **Analyst:** _____

Sample ID	Gastropod Species	Shell Height (cm)	Sex	Imposex Stage	Penis Length (mm)	Comments

General Comments: