Lower Duwamish Waterway Group

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

TECHNICAL MEMORANDUM: GASTROPOD PILOT SURVEY RESULTS 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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Table of Contents

Acronyms

ACRONYM	Definition
Ecology	Washington Department of Ecology
EPA	US Environmental Protection Agency
ERA	ecological risk assessment
GPS	global positioning system
LDW	Lower Duwamish Waterway
LDWG	Lower Duwamish Waterway Group
MHHW	mean higher high water
MLLW	mean lower low water
QAPP	Quality Assurance Project Plan
RI	Remedial Investigation
RM	river mile
RPS	relative penis size
TBT	tributyltin
Windward	Windward Environmental LLC
ww	wet weight

1.0 Introduction

This technical memorandum describes the results of the pilot survey of gastropods conducted in the Lower Duwamish Waterway (LDW) from June 16 to 21, 2004, based on the methods described in the gastropod pilot survey technical memorandum (Windward 2004c). Gastropods are of particular interest because of their sensitivity to tributyltin (TBT), a chemical present in LDW sediments. At sufficiently high tissue concentrations, TBT is known to cause the development of male sexual organs in females in some neo- and mesogastropod species[1] (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).

The pilot survey was designed to assess the presence of gastropods in the LDW, and to determine whether sufficient numbers of gastropods are present to allow for their collection and chemical analysis in a subsequent sampling effort. A secondary objective was to see if imposex occurred in gastropods found in the LDW and to determine the feasibility of evaluating the imposex endpoint for *in-situ*-exposed gastropods as part of the LDW ecological risk assessment. If gastropods are sufficiently abundant to warrant concern, but not sufficiently abundant to yield adequate tissue mass for chemical analysis, collection and analysis of tissue from a surrogate taxon would be considered. Alternatively, if gastropods are rare in the LDW, or if the imposex results suggest that gastropod reproduction would not be impaired, TBT might only be analyzed in the tissues of other benthic invertebrates collected under a "market basket" approach, with risks assessed for endpoints other than imposex (i.e., survival, growth, and reproduction).

The data from this survey will be used to support the Phase 2 ecological risk assessment (ERA) and remedial investigation (RI), as described in the work plan for the Phase 2 RI (Windward 2004b). Representatives from the US Environmental Protection Agency (EPA), the Washington Department of Ecology (Ecology), and the Lower Duwamish Waterway Group (LDWG) met on July 15, 2004 to discuss the pilot survey findings and answer the following questions:

- which organism tissue type would be collected for TBT analysis (i.e., gastropods, surrogate taxon, or market basket benthic invertebrate samples)
- where and how many tissue samples would be collected to cover the general range of TBT concentrations in sediment while collecting a sufficient mass of tissue for TBT analysis
- which methods would be most appropriate to collect the co-located tissue and surface sediment samples

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

Based on this meeting, it was determined that sufficient numbers and species of gastropods could be collected to directly assess risks to gastropods by assessing the imposex endpoint in field-collected gastropods, and that an additional survey for imposex in gastropods collected from subtidal areas of the LDW will be conducted in 2005. Therefore, neither gastropods, a surrogate taxon, nor market basket benthic invertebrates were collected for analysis of TBT during the pilot survey.

This report presents pilot survey methods in Section 2.0, results in Section 3.0, a summary and recommendations in Section 4.0, and a list of references in Section 5.0. It is supplemented by the following appendices:

- Appendix A. GPS Coordinates of Survey Locations
- Appendix B. Taxonomic Identification of Gastropods and Size Measurements
- Appendix C. Imposex Analysis Data Forms
- Appendix D. Field Notes from Gastropod Pilot Survey

This report is also supplemented by an HTML-based album of digital photographs taken during the field- sampling.

2.0 Pilot Survey Methods

On June 16, 17, 18, and 21, 2004, Windward Environmental (Windward) conducted a gastropod pilot survey as described in *Gastropod Pilot Survey of the Lower Duwamish Waterway* (Windward 2004c) . The survey covered intertidal areas in the lower 2.4 mi (i.e., river mile [RM] 0.0 to 2.4) and subtidal areas in the lower 3.8 mi (i.e., RM 0.0 to 3.8) of the LDW (Figure 2-1). Windward personnel present on this survey included Bob Complita, Derek Pelletier, Thai Do, Angelita Rodriquez, Helle Andersen, and Maryann Welsch. Kevin Li from King County supervised the deployment of the benthic sledge on June 16, 2004. Tom Gries from Ecology provided oversight on June 16 and June 18, 2004. The survey covered a range of historical TBT sediment concentrations in the LDW and a large range of habitats to ensure that potential gastropod habitats had been surveyed (Windward 2004a).

An overview of the methods employed in the intertidal and subtidal areas is presented in this section, as well as a summary of the taxonomic identification and imposex analysis methods used.

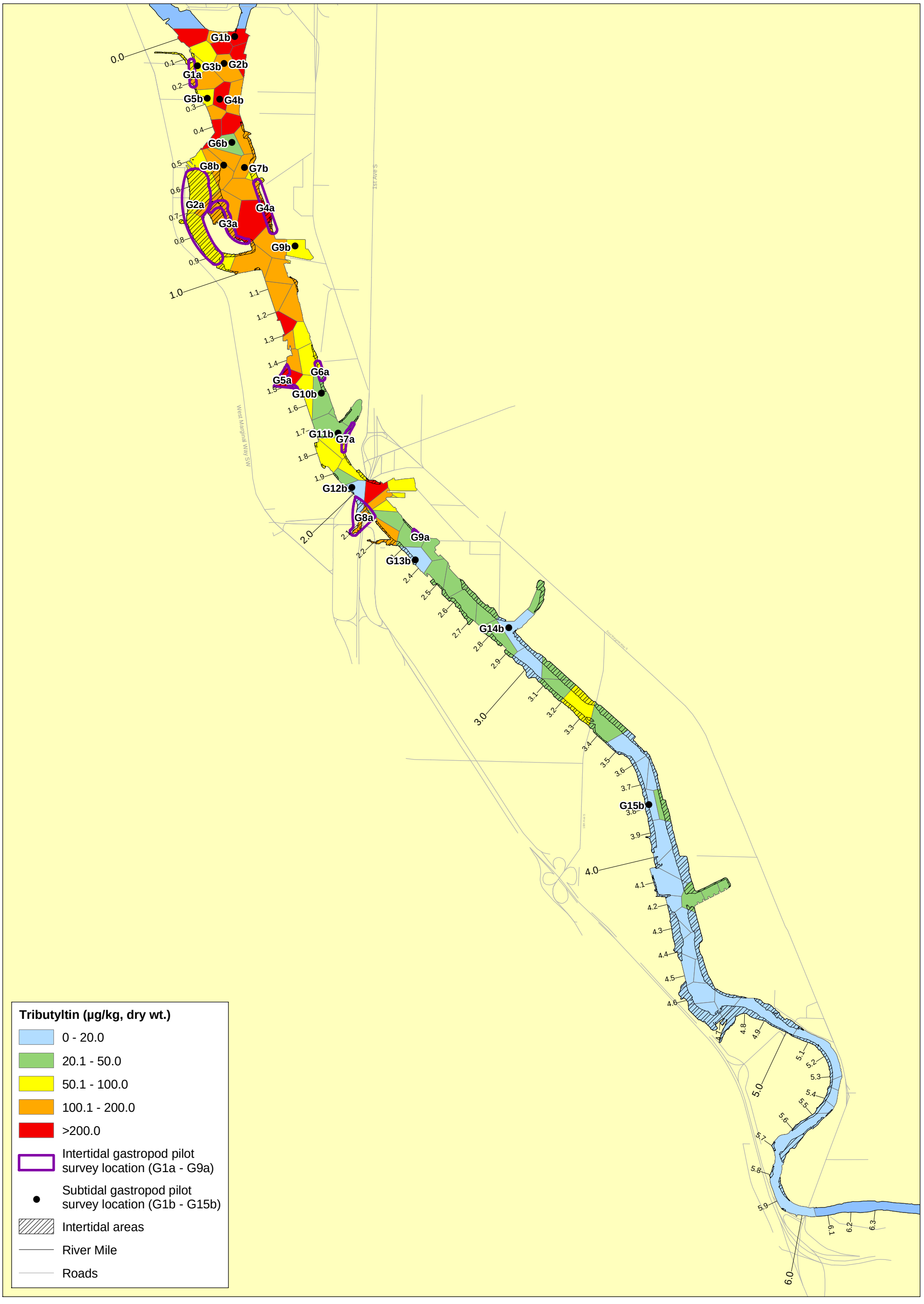

Figure 2-1. Gastropod pilot survey locations and tributyltin concentrations in LDW surface sediments by Thiessen polygon

Prepared by CEJ 06/01/04 Map 1392

Detection limits for concentrations reported as undetected were assigned a value of one-half the detection limit for the purpose of data aggregation.

W:\Projects\00-08-06_Duwamish_RI\data\gis\Gastropod Survey

2.1 INTERTIDAL GASTROPOD SURVEY

Windward conducted the intertidal portion of the gastropod survey during minus tides on June 16, 17, and 21, 2004. Nine intertidal areas from RM 0.1 to RM 2.4 were surveyed approximately two hours before and after a negative tide[2] to facilitate the search for organisms. Three intertidal areas were surveyed on each day, starting with the downstream areas and moving upstream (Table 2-1). At each intertidal area, a 100-m segment was placed parallel to the waterline, except at G3a where three 100-m segments were placed. All substrate types present in these areas were surveyed (i.e., rocks, sand, and mudflats). Each segment was divided into three sections perpendicular to the waterline up to mean higher high water (MHHW). The search at each area was performed by a team of three field crew members; one person walked along the waterline, one person walked midway up the beach, and the third person walked near MHHW[3]. Crew members covered 100 m at their designated elevation for a set amount of time (15 to 45 minutes per 100-m segment), depending on the complexity of the habitat surveyed (i.e., areas with numerous boulders and rocks were surveyed for a longer period of time than mudflat areas). Survey locations were documented with global positioning system (GPS) measurements at the start and end of each segment (see Appendix A for GPS coordinates for each segment).

Table 2-1. Intertidal gastropod pilot survey information

LOCATION ID	SURVEY DATE	GENERAL LOCATION DESCRIPTION	RIVER MILE	NUMBER OF 100-m TRANSECTS AND SURVEY TIME PER TRANSECT
G1a	June 16	north of Kellogg Island	0.1 – 0.2	1 transect in 45 min
G2a	June 17	west of Kellogg Island	0.5 – 0.9	1 transect in 30 min
G3a	June 16	east of Kellogg island	0.7 – 0.9	3 transects, 15 min each
G4a	June 16	east of Kellogg Island	0.6 – 0.9	1 transect in 30 min
G5a	June 17	western shoreline	1.4 – 1.5	1 transect in 30 min
G6a	June 17	eastern shoreline	1.4 – 1.6	1 transect in 30 min
G7a	June 21	Slip 2	1.7 – 1.9	1 transect in 30 min
G8a	June 21	west of Slip 3	2.0 – 2.1	1 transect in 30 min
G9a	June 21	south of Slip 3	2.2 – 2.4	1 transect[a] in 15 min

[a] Transect length at this location was approximately 50 m which was approximately the length of the intertidal area

During the intertidal survey of the 100-m segments for gastropods, each crew member collected representative epibenthic invertebrates such as mussels, isopods, and crabs in a separate plastic bag to assess the feasibility of collecting surrogate species. The collected epibenthic invertebrates were photographed as a group and returned to each survey area. After completing the gastropod search, the abundance of infaunal

[2] June 16th low tide at 11:00 AM (survey time 9:00 AM – 1:00 PM); June 17th low tide at 11:32 AM (survey time 9:30 AM – 1:30 PM); June 21st low tide at 1:05 PM (survey time 11:00 AM – 3:00 PM)

[3] All beaches except G4a were surveyed by three Windward crew members. At G4a, two Windward crew members performed the survey under the oversight of Tom Gries from Ecology

surrogate species in the finer-grained sediment areas of each location (i.e., not rocks, gravel, or shells) was assessed by placing a 0.1-m^2 stainless steel transect frame randomly at five locations between mean lower low water (MLLW) and MHHW within the first 100-m segment of each intertidal area. The invertebrates in the sediment were collected by digging the sediment within the frame to a depth of 10 cm and transferring it to a 1.0-mm mesh sieve. The sieve with the sediment was rinsed with LDW water to separate the organisms from sediment and organic matter. The infaunal organisms were photographed and returned to the digging site.

2.2 SUBTIDAL GASTROPOD SURVEY

Windward conducted the subtidal portion of the gastropod survey at 15 locations between RM 0.0 and RM 3.8 from June 16 to June 21, 2004. One subtidal location was surveyed on June 16, five locations on June 17, seven locations on June 18, and two locations on June 21 (Table 2-2). On the first day of the pilot survey, the performance of the preferred sampling device, a benthic sledge sampler, was assessed to determine whether it was reliable and retained relevant samples of organisms from the surface of the sediment. The assessment of the benthic sledge included ease of operation (i.e., whether the sledge gets stuck or lodged too deeply in the sediment or skims the surface of the sediment), the feasibility of performing tows of similar length and duration, and ability to collect epibenthic organisms. Test runs were 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 (i.e., ratio of length of rope deployed to water depth) of the tow rope and the deployment time of the sledge were determined. The benthic sledge was found to be relatively easy to operate, reliable, and able to collect gastropods. Hence, the sledge was used for the duration of the pilot survey.

Table 2-2. Subtidal gastropod pilot survey information

LOCATION ID	DATE	GENERAL LOCATION DESCRIPTION	RIVER MILE	TBT SED CONC (µg/kg dw)[a]	DEPTH (ft MLLW)	DISTANCE FROM TARGET LOCATION (m)	ACCEPTABLE TOWS/ TOTAL # TOWS	DURATION[b]	SCOPE[b]
G1b	6/17	south of southern tip of Harbor Island	0.0	358	-26.8	6.8-12.5	5/5	1 min	1:4
G2b	6/17	south of southern tip of Harbor Island	0.1	144	-39.6	3.8-13.1	5/5	1 min	1:4
G3b	6/16	south of southern tip of Harbor Island	0.1	94	-12.4	16.6-41.2	5/5	30 sec or 1 min	1:4 or 1:5
G4b	6/17	north of Duwamish Diagonal outfall	0.3	350	-39.8	2.7-14.3	5/6	1 min	1:4
G5b	6/17	north of Duwamish Diagonal outfall	0.3	53	-22.3	9.3-24.1	5/5	1 min	1:4, 1:5, or 1:6

Location ID	Date	General Location Description	River Mile	TBT Sed conc (µg/kg dw)[a]	Depth (ft MLLW)	Distance from Target Location (m)	Acceptable Tows/ Total # Tows	Duration[b]	Scope[b]
G6b	6/17	near Duwamish Diagonal outfall	0.5	34	-34.2	7.3-9.7	5/5	1 min	1:4
G7b	6/18	south of Duwamish Diagonal outfall	0.6	180	-26.7	6.2-11.4	5/7	30 sec, 40 sec or 1 min	1:4
G8b	6/18	south of Duwamish Diagonal outfall	0.6	117	-17.0	2.6-10.7	5/8	1 min	1:3.5 or 1:4
G9b	6/18	Slip 1	0.9	100	-27.9	3.8-11.9	5/6	45 sec or 1 min	1:3, 1:35, or 1:4
G10b	6/18	north of Slip 2	1.6	25	-22.7	4.3-42.9	5/7	40 sec or 1 min	1:4
G11b	6/18	mouth of Slip 2	1.8	42	-17.4	4.3-11.4	5/6	1 min	1:3, 1:3.5, or 1:4
G12b	6/18	west of slip 3	2.0	20	-13.9	3.3-59.9[c]	5/7	1 min	1:4
G13b	6/18	south of West Michigan outfall	2.4	1	-6.7	105.3-147.1[c]	5/6	1 min	1:3 or 1:4
G14b	6/21	mouth of Slip 4	2.8	6	-16.8	30.4-38.1	5/6	30 sec or 1 min	1:3 or 1:4
G15b	6/21	south of T117	3.8	12	-4.0	5.2-18.7	5/8	1 min	1:3 or 1:4

[a] TBT concentration in a single grab sediment sample previously collected at target location, as reported in the Phase 1 RI (Windward 2004b)

[b] The scope and/or the duration of the tow were changed between repeat tows if the sledge was empty or completely full of sediment when it was retrieved or if it was caught during the tow

[c] Location was moved as a result of obstructions (e.g., piling and piers) in the water

Five acceptable tows were performed within 10-50 m of each target GPS location specified in the pilot survey methods memorandum (Windward 2004c) to estimate catch variability among the tows of the sledge. Unacceptable tows consisted of the bag retrieved empty or completely full of sediment.[4] GPS was used to identify the location of each sledge deployment (see Appendix A for GPS coordinates of each tow). All tows were performed within 41 m of the targeted location, except at G12b and G13b, where the sampling location was moved from 48 to 147 m from the targeted location due to obstructions in the water or difficulties making acceptable tows. In accordance with the QAPP, all tows were performed relatively close to the GPS location even when no or few gastropods were observed in the first tow. In general, the scope was 1:4 and the duration of each tow was 1 minute (Table 2-2). At the completion of the designated pulling time, the sledge was slowly winched to the surface of the water, and the contents of the bag were sieved by moving the sledge back and forth through the water. When the contents had been sieved as much as possible in the bag, the sledge was lifted on board and the contents in the bag were transferred into a 1.0-mm

[4] Completely full bags may not be representative of the area being sampled because it is not possible to determine if the bag had overflowed, thereby losing some of the sample. In addition, full bags were extremely heavy and difficult to handle, and would require a very long time to process.

mesh sieve. The contents were further sieved in the 1.0-mm screen, after which the gastropods were picked from the sieve and placed into a wide-mouth glass jar partially filled with Elliott Bay surface water,[5] and stored on ice. Other organisms in the sieve were identified, photographed, and returned to the sampling site.

2.3 TAXONOMIC IDENTIFICATION

Gastropods were identified in two rounds because of the compressed time schedule of the survey and time availability of Dr. Kohn, the gastropod expert at the University of Washington, who received the specimens for imposex analyses. All gastropods collected as part of this pilot survey were identified to species or genus by Dr. Fukuyama, the expert gastropod taxonomist. All gastropods with shell heights larger than approximately 1 cm were to be analyzed for imposex by Dr. Kohn. Gastropods were placed in separate vials (i.e., one vial for > 1 cm and one vial for < 1 cm at each station) with saltwater from Elliott Bay to keep gastropods alive prior to imposex analyses. Dr. Kohn requested live gastropods with a shell height larger than 1 cm for the imposex analysis because he did not know which gastropod species would be collected and he was unsure if he would be able to see the anatomy of imposexed females in smaller specimens. After completion of the imposex analyses, Dr. Kohn stated that he would be able to evaluate specimens smaller than 1 cm. However, he did not have time available to examine any additional specimens. He did not specify a smaller size threshold, but suggested that if additional collection occurred in the future he should be given all gastropods.

Gastropods identified in Round 1 consisted of 65 specimens with a shell height of approximately 1 cm or larger collected June 16, 17, and 18. Prior to the Round 1 taxonomic identification, the shell heights of all gastropods collected June 16 and 17, 2004, were measured in the Windward laboratory and all individuals with shell heights larger than approximately 1 cm were placed in separate vials. The shell heights of gastropods collected on June 18 were not measured prior to the 7:30 PM June 18 delivery to Dr. Fukuyama for taxonomic identification because of the limited time between the field effort and delivery of the gastropods to Dr. Fukuyama. Instead, gastropods collected on June 18 were visually inspected and the *Nassarius mendicus* gastropods and some of the *Astyris gausapata* gastropods that appeared to be larger than 1 cm were included in the delivery to Dr. Fukuyama so they could also be assessed for imposex by Dr. Kohn.

While in Dr. Fukuyama's possession, the gastropods were kept in a cooler with ice. Dr. Fukuyama identified them to species and/or genus and returned them to Windward at 10:55 AM on June 19, 2004. This process of separating larger gastropods and having

[5] Surface water was obtained from Elliott Bay so that the salinity would be closer to the LDW subtidal locations that were sampled. In the LDW, freshwater from the Duwamish River is generally found at the surface, whereas the saline water from Elliott Bay that moves into the LDW via tidal exchange is closer to the bottom because it is denser than freshwater.

them identified over the weekend ensured that Dr. Kohn received only neo- and mesogastropods at the requested size range. The identified gastropods were kept in jars with saltwater from Elliott Bay, covered with netting, and stored in a sample refrigerator until they were delivered to Dr. Kohn at 10:25 AM on June 21, 2004 for imposex analyses.

All remaining gastropods collected during the survey were identified in Round 2. Specifically, gastropods identified in Round 2 included smaller gastropods collected June 16 and 17 (shell height < 1 cm), all gastropods collected June 18 except those separated out the evening of June 18, and all gastropods collected June 21. The gastropods were delivered to Dr. Fukuyama on June 22, 2004, and all identification was completed by June 25, 2005.

2.4 IMPOSEX ANALYSES

Imposex analyses of gastropods collected from locations G1b-G10b and G12b on June 16, 17, and 18, 2004 were performed by Dr. Kohn on June 21 and 22, 2004.[6] These locations represented a range of TBT concentrations in sediment (34 to 358 µg/kg dw), including the two locations with the highest TBT concentrations (Table 2-2, Figure 2-1). The analysis commenced by cracking the shell with a hammer and determining the gender of the animal. The length of the male penises was measured in half of the males before all of the males were returned to the sampling jar. The jars were stored in a cooler with ice. The females were examined for imposex using the approaches reported in Oehlmann et al. (1991) and Spence et al. (1990). Oehlmann et al. (1991) determined the imposex stage based on the presence of male reproductive organs in females, including vas deferens[7] and penis. Table 2-3 shows the imposex stages used for this evaluation. Because the vas deferens could not be seen, penis length was measured to assess the level of imposex using the relative penis size (RPS) approach (Gibbs et al. 1988). The RPS index is calculated using the following equation:

$$\text{RPS index} = (\text{mean length of female penis}^3 / \text{mean length of male penis}^3) \times 100$$

After completion of the imposex analyses, the gastropods were returned to Windward at 1:05 PM on June 22, 2004, and stored in a sample freezer.

[6] Dr. Kohn did not receive the gastropods identified by Dr. Fukuyama in Round 2 because of the limited time he was available.

[7] A sperm-carrying duct

Table 2-3. 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: 1) larger penis with a penis duct, or 2) two sections of vas deferens, or 3) both a penis and a vas deferens section
3	development of either: 1) larger penis with vas deferens section, or 2) a complete vas deferens, or 3) 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

3.0 Results

3.1 INTERTIDAL GASTROPOD SURVEY

No gastropods were found during the survey of the nine intertidal areas. Other larger epibenthic invertebrates observed during the survey included barnacles, crabs, isopods, amphipods, and mussels. Very few infaunal invertebrates were observed in the field after sieving with a 1 mm sieve; the most abundant invertebrate was the amphipod *Corophium* sp. The invertebrates observed at each intertidal area are presented in Table 3-1.

Table 3-1. Intertidal gastropod pilot survey observations

LOCATION ID	GENERAL LOCATION DESCRIPTION	RIVER MILE	NUMBER OF GASTROPODS	EPIBENTHOS	INFAUNA IN FIVE 0.1-m^2 AREAS
G1a	north of Kellogg Island	0.1 – 0.2	0	on or under rocks: *Hemigrapsus* sp, isopods including *Idotea* sp., *Mytilus* sp., *Balanus* sp., one limpet, amphipods sand and mud flats: no epibenthos	numerous *Corophium* sp., few *Macoma baltica*
G2a	west of Kellogg Island	0.5 – 0.9	0	on or under rocks: *Hemigrapsus* sp., *Mytilus* sp., *Balanus* sp., amphipods sand and mud flats: no epibenthos	few *Corophium* sp., five *Macoma baltica*
G3a	east of Kellogg island	0.7 – 0.9	0	on or under rocks: *Hemigrapsus* sp., *Idotea* sp., *Mytilus* sp., *Balanus* sp., numerous amphipods sand and mud flats: no epibenthos	few *Corophium* sp., two *Macoma baltica*, few polychaetes

Location ID	General Location Description	River Mile	Number of Gastropods	Epibenthos	Infauna in five 0.1-m^2 areas
G4a	east of Kellogg Island	0.6 – 0.9	0	on or under rocks: *Hemigrapsus* sp., *Idotea* sp., *Mytilus* sp., *Balanus* sp., amphipods, numerous larger polychaetes sand and mud flats: no epibenthos	few *Corophium* sp., two *Macoma baltica*
G5a	western shoreline	1.4 – 1.5	0	on or under rocks: *Hemigrapsus* sp., *Idotea* sp., *Mytilus* sp., *Balanus* sp., amphipods sand and mud flats: no epibenthos	numerous *Corophium* sp., few *Macoma baltica*, few polychaetes
G6a	eastern shoreline	1.4 – 1.6	0	on or under rocks: *Hemigrapsus* sp., *Idotea* sp., *Mytilus* sp., *Balanus* sp., amphipods sand and mud flats: no epibenthos	numerous *Corophium* sp., three *Macoma baltica*
G7a	Slip 2	1.7 – 1.9	0	on or under rocks: *Hemigrapsus* sp., *Mytilus* sp., *Balanus* sp., isopods, amphipods sand and mud flats: no epibenthos	few *Corophium* sp.
G8a	west of Slip 3	2.0 – 2.1	0	on or under rocks: *Hemigrapsus* sp., *Mytilus* sp., *Balanus* sp., isopods, amphipods sand and mud flats: no epibenthos	few *Corophium* sp., 16 *Macoma baltica*, three polychaetes
G9a	south of Slip 3	2.2 – 2.4	0	on or under rocks: *Hemigrapsus* sp., *Mytilus* sp., *Balanus* sp., isopods, amphipods sand and mud flats: no epibenthos	one *Macoma baltica*[a]

[a] Space was limited; fewer than five complete 0.1-m^2 frames were placed and a depth of 10 cm was not always achieved.

3.2 Subtidal Gastropod Survey

A total of 739 gastropods were collected in subtidal areas of the LDW. Gastropods were present at all 15 subtidal locations with a mean number of gastropods per tow at each location ranging from less than 1 to 48 (Table 3-2). The majority of the gastropods were collected within the first river mile with only 12 gastropods collected further upstream. Other larger invertebrates observed during the survey included crabs, sea pens, shrimp, bivalves, and others. The invertebrates observed at each subtidal location are presented in Table 3-2.

Table 3-2. Subtidal gastropod pilot survey observations

Location ID	River Mile	Depth (ft MLLW)	Historical TBT Sed conc (µg/kg dw)[a]	Substrate Type in Sledge	Total Number of Gastropods	Mean Number of Gastropods per Tow (± stdev)	Other Invertebrates
G1b	0.0	-26.8	358	sand and debris	107	21 ± 12	shrimp, one slipper snail, one sea pen
G2b	0.1	-39.6	144	sand	239	48 ± 36	shrimp, one *Cancer gracilis*
G3b	0.1	-12.4	94	sand	45	9 ± 12	numerous *Clinocardium* sp. one hermit crab, one spider crab, slipper snails, shrimps, *Balanus* sp., one *Mya arenaria,* starfish, one chiton
G4b	0.3	-39.8	350	mud	77	15 ± 9	two *Cancer gracilis*, one urchin,
G5b	0.3	-22.3	53	sand	4	0.8 ± 0.8	one hermit crab
G6b	0.5	-34.2	34	mud, wood debris, dark sand	74	15 ± 17	shrimp, hermit crabs
G7b	0.6	-26.7	180	rocks and gravel	5	1 ± 1	shrimp
G8b	0.6	-17.0	117	sand and mud	153	31 ± 23	numerous small bivalves
G9b	0.9	-27.9	100	mud	23	5 ± 3	numerous small bivalves
G10b	1.6	-22.7	25	sand and lots of organic matter	4	0.8 ± 0.4	numerous small bivalves, shrimp, one sea pen, one sea anemone, some amphipods, numerous small polychaetes, two opisthobranchs
G11b	1.8	-17.4	42	sand and lots of organic matter	1	0.2 ± 0.4	several small bivalves, shrimp, some amphipods, numerous small polychaetes
G12b	2.0	-13.9	20	debris and organic matter	1	0.2 ± 0.4	*Balanus* sp., some amphipods, numerous small polychaetes
G13b	2.4	-6.7	1	organic matter and silt	3	0.6 ± 0.9	*Clinocardium* sp., *Macoma baltica*, small bivalves, shrimp, some amphipods, numerous small polychaetes
G14b	2.8	-16.8	6	small shells and silt	2	0.4 ± 0.5	bivalves, polychaetes
G15b	3.8	-4.0	12	small shells and silt	1	0.2 ± 0.4	polychaetes, amphipods, mysids, bivalves, *Corophium* sp.

[a] TBT concentration in a single grab sediment sample previously collected at target location, as reported in the Phase 1 RI (Windward 2004b)

3.3 TAXONOMIC IDENTIFICATION

The 739 gastropods collected during the pilot survey were distributed over 11 taxa (species or genus). Of these 11 taxa, three are in the order Neogastropoda and five are in the order Mesogastropoda (Table 3-3). The most abundant species was the neogastropod *Astyris gausapata* (formerly *Mitrella goldii*) which accounted for 86% of all collected gastropods and was found at 11 locations. This species was found at all subtidal locations up to RM 1.0 (Table 3-4). Further upstream, only two specimens of *A. gausapata* were collected, one at G10b and one at G12b. The next most common neogastropod, *Nassarius mendicus*, which accounted for 4% of all the collected gastropods, was also primarily found in the area below RM 1.0; only one specimen was collected further upstream at G10b. This species was found at 9 locations (Table 3 3). Mesogastropods or other neogastropods were collected in very low numbers (1-6) at locations G1b through G11b (Table 3-4), and each species was only present at a few locations (Table 3-3). Only one genus, *Turbonilla* sp. (not a neo- or mesogastropod), was found throughout the waterway (but not at every location) from RM 0.0 to RM 3.8.

Table 3-3. Gastropod taxa and total abundances

SPECIES/GENUS	ORDER	TOTAL NUMBER COLLECTED	MEAN SHELL HEIGHT (cm) (+stdev)	LOCATIONS
Alvania compacta	Mesogastropoda	5	0.22 ± 0.03	G1b, G8b
Astyris gausapata	Neogastropoda	633	0.78 ± 0.19	G1b, G2b, G3b, G4b, G5b, G6b, G7b, G8b, G9b, G10b, G12b
Cryptonatica affinis	Mesogastropoda	1	0.51	G11b
Euspira sp.	Mesogastropoda	6	0.28 ± 0.10	G2b, G9b, G10b
Kurtzia arteaga	Neogastropoda	5	0.87 ± 0.20	G3b, G4b, G6b
Lacuna vincta	Mesogastropoda	2	0.70 ± 0.01	G7b, G8b
Nassarius mendicus	Neogastropoda	27	1.20 ± 0.14	G1b, G2b, G3b, G4b, G5b, G6b, G7b, G8b, G10b
Odostomia sp.	Pyramidellacea	14	0.34 ± 0.09	G1b, G2b, G3b, G6b, G9b
Rictaxis punctocaelatus	Cephalaspidea	1	0.34	G9b
Trichotropis cancellata	Mesogastropoda	2	0.61 ± 0.36	G2b, G5b
Turbonilla sp.	Pyramidellacea	43	0.52 ± 0.19	G1b, G2b, G6b, G8b, G9b, G13b, G14b, G15b

Table 3-4. Neo- and mesogastropods collected at each subtidal location

LOCATION ID	RIVER MILE	TOTAL NUMBER OF GASTROPODS	TOTAL NUMBER OF *NASSARIUS MENDICUS*	TOTAL NUMBER OF *ASTYRIS GAUSAPATA*	TOTAL NUMBER OF OTHER NEO- AND MESOGASTROPODS
G1b	0.0	107	7	69	2
G2b	0.1	239	8	222	2

Location ID	River Mile	Total Number of Gastropods	Total Number of *Nassarius mendicus*	Total *Number of Astyris gausapata*	Total Number of Other Neo- and Mesogastropods
G3b	0.1	45	1	42	1
G4b	0.3	77	1	73	3
G5b	0.3	4	1	2	1
G6b	0.5	74	5	66	1
G7b	0.6	5	1	3	1
G8b	0.6	153	2	140	4
G9b	0.9	23	0	14	3
G10b	1.6	4	1	1	2
G11b	1.8	1	0	0	1
G12b	2.0	1	0	1	0
G13b	2.4	3	0	0	0
G14b	2.8	2	0	0	0
G15b	3.8	1	0	0	0

3.4 IMPOSEX ANALYSIS

Dr. Kohn received 65 neogastropods greater than 1 cm in shell height, including 39 *Astyris gausapata* (formerly *Mitrella gouldii*) and 26 *Nassarius mendicus*.[8] In addition, Dr. Kohn received two *Turbonilla* sp. from the order Pyramidellacea. These specimens were given to Dr. Kohn to confirm the identification made by Dr. Fukuyama, but these gastropods were not assessed for imposex because they are not neo- or mesogastropods.

Fourteen out of 39 specimens of *A. gausapata* with shell heights greater than 1 cm were examined for imposex. The 14 individuals examined were collected at locations G1b, G2b, G3b, and G4b. The highest TBT concentrations in historical sediment samples collected from these locations were at G1b and G4b (approximately 350 µg/kg dw, Table 2-2). Four of the *A. gausapata* examined were male and 10 were female. None of the female *A. gausapata* showed evidence of imposex. Due to limitations in Dr. Kohn's available time and because no imposex was seen in gastropods collected from areas with the highest TBT concentration in sediment, Dr. Kohn did not examine the remaining 25 specimens of *A. gausapata* collected at locations G5b, G6b, G7b, G8b, G9b, G10b, and G12b.

Twenty-four out of 26 specimens of *N. mendicus*[9] sent to Dr. Kohn were examined for imposex. These individuals were collected at locations G1b, G2b, G3b, G4b, G5b, G6b,

[8] One additional specimen of *N. mendicus* from location G8b was identified later when all the remaining gastropods were identified by Dr. Fukuyama; hence, this specimen was not received by Dr. Kohn. Also because shell heights of all gastropods could not be measured before delivering them to Dr. Fukuyama, a few *Kurtzia arteaga* gastropods of approximately 1 cm were not given to Dr. Kohn for imposex analysis.

[9] All individuals were $\geq$ 1 cm

G7b, G8b, and G10b. Due to limitations in Dr. Kohn's available time, two of the eight specimens collected at location G2b were not analyzed. Fourteen of the *N. mendicus* examined were male and 10 were female.

The imposex analysis performed according to Oelhmann et al (1991) includes six stages of imposex (Table 2-3). Complete sterilization is associated with stages 5 and 6. Stage 4 is generally referred to as transitional, and stages 1 through 3 are described as early stages. The degree of imposex in all female *N. mendicus* examined was similar to Stage 2 of Oehlmann et al. (1991), except that in all cases penises were present but no vasa deferentia[10] were observed. The RPS index was calculated using the data presented in Table 3-5. Penis length was measured in 7 of the 14 male specimens. Dr. Kohn began measuring the male penis length once it became clear that the vas deferens could not be observed in the female specimens with penises. Because imposex does not apply to males, the mean penis length over all 5 locations where male penis length was measured was used to calculate the RPS index for each location.

Table 3-5. *Nassarius mendicus* penis lengths and shell heights

Location ID	Female Penis Length (mm)	Female Shell Height (cm)	Male Penis Length (mm)	Male Shell Height (cm)
G1b	Specimen 1 - 0.86	Specimen 1 - 1.1	Specimen 1 - nm Specimen 2 - nm Specimen 3 - nm Specimen 4 - nm Specimen 5 - nm Specimen 6 - nm	Specimen 1 - nm Specimen 2 - nm Specimen 3 - nm Specimen 4 - nm Specimen 5 - nm Specimen 6 - nm
G2b	Specimen 1 - 0.83 Specimen 2 - 1.8 Specimen 3 - 1.8 Specimen 4 - 1.8	Specimen 1 - 1.2 Specimen 2 - 1.2 Specimen 3 - 1.3 Specimen 4 - 1.1	Specimen 1 - 5.0 Specimen 2 - nm	Specimen 1 - 1.5 Specimen 2 - nm
G3b	Specimen 1 - 1.8	Specimen 1 - 1.1	na	na
G4b	na	na	Specimen 1 - 4.6	Specimen 1 - 1.1
G5b	na	na	Specimen 1 - 5.0	Specimen 1 - 1.4
G6b	Specimen 1 - 0.09 Specimen 2 - 1.2 Specimen 3 - 0.8	Specimen 1 - 1.35 Specimen 2 - 1.32 Specimen 3 - 1.28	Specimen 1 - 8.7 Specimen 2 - 8.3	Specimen 1 - 1.37 Specimen 2 - 1.19
G7b	na	na	Specimen 1 - 7.1	Specimen 1 - 1.08
G8b	Specimen 1 - 1.1	Specimen 1 - 1.11	na	na
G10b	na	na	Specimen 1 - 9.1	Specimen 1 - 1.35

na – not available; this gender was not collected at this location

nm – not measured

[10] Plural of vas deferens

RPS indices at the locations where female *N. mendicus* were collected and analyzed are presented in Table 3-6. All indices were 1.8% or less. Spence et al. (1990) states that, in general, sterile females are absent at RPS indices below 5%, between 5 and 40% the percentage of sterility increases, and at RPS indices exceeding 40%, most or all females are sterile.

Table 3-6. TBT concentrations in sediment and *Nassarius mendicus* RPS indices

Location ID	TBT Concentration in Sediment[a] (µg/kg dw)	Mean Female Penis Length (mm) (±stdev) (number measured)	Mean Male Penis Length (mm) (number measured)	RPS Index
G1b	358	0.86 (1)		0.2
G2b	144	1.56 ± 0.49 (4)		1.2
G3b	94	1.8 (1)		1.8
G6b	34	0.70 ± 0.56 (3)		0.1
G8b	117	1.1 (1)		0.4
G2b-G10b			6.83 (7)	

[a] TBT concentration in sediment at a single grab sample previously collected at target location, as reported in the Phase 1 RI (Windward 2004b)

3.5 Biomass Estimate

One of the objectives for the gastropod pilot survey was to assess the feasibility of collecting sufficient tissue mass for TBT analyses. Gastropods evaluated for imposex were weighed by Dr. Kohn; the other specimens were weighed at the Windward laboratory. The total weights of gastropods (all species combined), including shell, per tow, are given in Table 3-7. Because any chemical analysis of gastropod tissue would exclude the shell, understanding the relationship between total weight (including shell) and tissue weight is desirable. No data could be found that related the weight of a gastropod with shell to the tissue mass. Tokeshi et al. (2000) related gastropod shell height to the tissue mass. Using the relationship described in Tokeshi et al. (2000), the tissue mass estimated from shell heights was as much as 80% of the measured total weights. This estimate seemed high, given the laboratory observations of shell size and thickness.

Table 3-7. Total gastropod weights per tow at each location

Location ID	Total Weight (g ww, including shell) of All Gastropods Collected per Tow				
	Tow 1	Tow 2	Tow 3	Tow 4	Tow 5
G1b	0.66	0.22	0.11	2.65	<0.1
G2b	5.98	<0.1	1.81	6.94	1.71
G3b	na	na	na	2.62	2.5
G4b	1.06	0.21	2.65	<0.1	0.23
G5b	<0.1	0.39	na	na	<0.1
G6b	3.11	1.38	0.96	<0.1	0.7

Location ID	Total Weight (g ww, including shell) of All Gastropods Collected per Tow				
	Tow 1	Tow 2	Tow 3	Tow 4	Tow 5
G7b	<0.1	0.2	na	na	<0.1
G8b	8.7	1.2	2.6	5.0	2.1
G9b	0.2	0.9	na	0.3	<0.1
G10b	na	<0.1	<0.1	<0.1	0.41
G11b	na	na	na	na	<0.1
G12b	na	na	na	<0.1	na
G13b	na	na	<0.1	na	<0.1
G14b	na	na	na	<0.1	<0.1
G15b	<0.1	na	na	na	na

na: not applicable; no gastropods collected in this tow

As an alternative way to estimate the level of effort it would take to collect 2 g[11] (ww) of gastropod tissue mass, which is the required amount specified in the benthic invertebrate QAPP (Windward 2004a), the number of tows needed per location was calculated, based on the assumption that the tissue mass was 20, 30, 40, or 50% of the total weight (ww), including shell (Table 3-8). Sufficient tissue mass could be collected at the following locations based on these estimates and a field effort of less than 15 tows per location: G1b through G4b, G6b, and G8b. The sediment TBT concentrations at these locations ranged from 34 to 358 µg/kg dw. Gastropod abundances at all other locations were so low that an infeasible amount of effort would be required to collect a sufficient mass of gastropod tissue.

Table 3-8. Estimated number of tows to collect 2 g of gastropod tissue mass based on the pilot survey

Location ID	Number of Tows Required if the Tissue Mass Represents the Following Percentages of the Total Weight (ww)			
	20% of total weight	30% of total weight	40% of total weight	50% of total weight
G1b	14	9	7	5
G2b	3	2	2	1
G3b	10	7	5	4
G4b	12	8	6	5
G5b	128	85	64	51
G6b	8	5	4	3
G7b	250	167	125	100
G8b	3	2	1	1
G9b	36	24	18	14
G10b	122	81	61	49
G11b	na	na	na	na
G12b	na	na	na	na
G13b	na	na	na	na

[11] Including QC samples

Location ID	Number of Tows Required if the Tissue Mass Represents the Following Percentages of the Total Weight (ww)			
	20% of total weight	30% of total weight	40% of total weight	50% of total weight
G14b	na	na	na	na
G15b	na	na	na	na

na: not applicable; it is not feasible to collect sufficient gastropod tissue mass for analysis of TBT in tissue

4.0 Summary and Recommendations

The results from the gastropod pilot survey are summarized as follows:

- No gastropods were found in intertidal areas over a range of sediment TBT concentrations of 31 to 216 µg/kg dw[12] and across various substrate types (e.g., rocks, mudflats, etc.)
- Gastropods were found throughout the subtidal locations surveyed, although their abundances were much lower in the upstream portions of the LDW surveyed
- Neogastropods were found from the far northern portion of the LDW upstream to location G12b at RM 2.0. Mesogastropods were found in low numbers throughout the waterway up to RM 1.8
- Eight neo- or mesogastropod species were found; two neogastropod species were large enough to assess for imposex (> 1 cm)
- No signs of imposex were found in female *A. gausapata,* which was the most abundant gastropod in the LDW, over a range of sediment TBT concentrations of 94 to 358 µg/kg dw
- Stage 2 imposex was observed in female *N. mendicus* collected at locations G1b, G2b, G3b, G6b, and G8b
- The RPS indices calculated for the 5 locations where female *N. mendicus* were collected were all well below thresholds associated with sterility in female neogastropods
- If TBT analyses were to be performed on gastropod tissue from the LDW, collection of 2 g of gastropod tissue may be feasible at 6 of the 15 subtidal locations. If the required biomass was lowered to 500 mg (with potentially higher detection limits), collection of sufficient gastropod tissue may be feasible at 7 subtidal locations.

Based on the pilot survey results, LDWG and EPA/Ecology have reached the following conclusions:

[12] TBT concentration in sediment at a single grab sample previously collected at target location, as reported in the Phase 1 RI (Windward 2004b).

- In intertidal areas, no additional work to assess TBT and gastropods is warranted, primarily because gastropods do not appear to be present in intertidal areas.
- In subtidal areas, additional gastropod collection is warranted to further directly assess the imposex endpoint in gastropods in the LDW. Sampling will be conducted over a mutually agreed area, and the neo- and mesogastropod species collected will be examined for imposex by Dr Kohn. The gastropod samples collected in the second sampling will all be delivered to Dr. Kohn for imposex analysis. It is expected that the minimum size limit for the determination of imposex will be smaller than 1 cm, that minimum size is as yet unknown. The number of individuals per species assessed will be determined in consultation with EPA and Ecology. Specific sampling and analysis details will be provided in a draft methods memorandum to be submitted to EPA and Ecology on June 17, 2005.
- Prior to determining the number of tows and sampling locations for this second gastropod collection, LDWG, EPA and Ecology will collectively review recent TBT surface sediment and any available TBT porewater data. Thus, the second gastropod sampling is being postponed so that the exposure regime (relative to TBT) can be better characterized.
- Tissue analyses of gastropods from the LDW are not warranted because the assessment endpoint of concern for gastropods (imposex) was determined in the pilot study to be directly measurable, and will be further assessed in 2005.
- As described in the benthic invertebrate QAPP (Windward 2004a), market basket benthic invertebrate tissue samples will be analyzed for TBT in intertidal and subtidal areas to assess TBT risks to other benthic invertebrates based on survival, growth, and reproduction endpoints. The imposex results from this pilot survey and the survey to be conducted in 2005 will be discussed in the ERA to address risks to gastropods from exposures to TBT.

5.0 References

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