# Lower Duwamish Waterway Group

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

# TECHNICAL MEMORANDUM: 2005 GASTROPOD IMPOSEX STUDY 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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## Acronyms

| ACRONYM | Definition |
|---|---|
| dw | dry weight |
| Ecology | Washington State 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 |
| MLLW | mean lower low water |
| RI | remedial investigation |
| RM | river mile |
| RPS | relative penis size |
| TBT | tributyltin |
| Windward | Windward Environmental LLC |

# 1.0 Introduction

This technical memorandum describes the results of the gastropod imposex study conducted in the Lower Duwamish Waterway (LDW) from August 8 to 11, 2005, based on the methods described by Windward Environmental LLC (2004b). A pilot gastropod survey was conducted in 2004 to assess the abundance of gastropods in the LDW, evaluate the prevalence of imposex among LDW gastropods, and determine the feasibility of evaluating the imposex endpoint for gastropods exposed in situ to elevated concentrations of tributyltin (TBT) in sediment. Based on findings from the 2004 pilot survey (Windward 2004c), the Lower Duwamish Waterway Group (LDWG), the Washington State 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.

The data from this study and the 2004 pilot survey 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 2004a). Section 3.1.5 of the work plan identified the need for additional data to assess risks from 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 report presents the study methods in Section 2.0, the results in Section 3.0, a summary 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 sampling locations
- Appendix B – Dr. Kohn's imposex report and data forms
- Appendix C – Collection forms and field notes
- Appendix D – Chain-of-custody forms

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

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

# 2.0 Imposex Study Methods

On August 8, 9, 10, and 11, 2005, Windward collected gastropods from the LDW as described in a technical memorandum that present the study methods (Windward 2004b). Gastropods were collected at six subtidal locations in the lower 1.3 mi (i.e., river mile [RM] 0.0 to RM 1.3) of the LDW (Figures 2-1 and 2-2). The six collection locations targeted areas with higher TBT sediment concentrations based on historical sediment data and sediment data from both the benthic invertebrate sampling event conducted in August-September 2004 and the surface sediment sampling events conducted in January-March 2005. Surface sediment data from these sampling events were reported by Windward (2005a, b, c). Dr. Kohn received all gastropods collected and assessed the meso- and neogastropods for imposex at his laboratory at the University of Washington.

An overview of the methods employed at the subtidal locations and in the imposex analyses is presented in this section.

## 2.1 GASTROPOD COLLECTION METHODS

Gastropods were collected using a benthic sledge at one to three locations on each of the four days (Table 2-1), for a total of six target locations. Ten acceptable tows were performed at each target location, as specified in the technical memorandum presenting study methods (Windward 2004b). A global positioning system (GPS) was used to identify the location of each sledge deployment and retrieval (see Appendix A for GPS coordinates of each tow). The actual depths at time of sampling were recorded with a depth sounder and later corrected to mean lower low water (MLLW). The scope[2] ranged from 1:2 to 1:6, except at G21b, where the scope was up to 1:14 because of the shallow water at the start of the tow. Depending on the substrate, the duration of each tow ranged from 30 seconds in very soft sediments to 2 minutes in hard-packed sand (Table 2-1). 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 mesh sieve. The contents were then sieved in the 1.0-mm screen, after which all gastropods were picked from the sieve and sorted into separate wide-mouth glass jars partially filled with water collected near the sediment surface at each sampling location.[3]

Gastropods were field-sorted by placing the most abundant species (*Astyris gausapata* and *Olivella* sp.) in separate jars for analysis by Dr. Kohn at his University of Washington laboratory. Other gastropod species were placed in a separate jar for each location. At the end of the sampling effort at each location, the numbers of gastropods were noted on the field forms, the numbers of jars were noted on the chain-of-custody form, and the jars

[2] Ratio of length of rope deployed to water depth

[3] The water was collected with a modified Schindler trap.

were placed on ice in a cooler. At the end of each day, the gastropods were hand-delivered to Dr. Kohn for imposex analysis. Field notes and chain-of-custody forms are presented in Appendices C and D, respectively.

## 2.2 IMPOSEX ANALYSIS METHODS

The gastropods were identified to species or genus by Dr. Kohn prior to the imposex analysis. Imposex analyses were conducted on meso- and neogastropods. The other gastropods collected in the LDW belong to genera that are hermaphroditic and, therefore, were not evaluated for imposex. Dr. Kohn performed the imposex analyses on live organisms after cracking the shell with a hammer and determining the gender of the animal. However, one of the evaluated species (*Olivella baetica*) produced large quantities of mucus, thereby obscuring the sex organs. This species was therefore euthanized in boiling water for two minutes before its shell was cracked, and it was transferred to cold water and then to 70% alcohol to denature the mucus before imposex was assessed.

## Table 2-1. Gastropod collection information

| Gastropod Collection Target Location ID | Date | General Location Description | River Mile | Depth (ft MLLW) | | Distance from Target Location (m)[b] | | Duration[c] | Scope[c] | Location ID for Surface Sediment Samples[d] | TBT Sediment Concentration as ION (µg/kg dw)[e] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Start of Tows[a] | End of Tows[a] | Start of Tows[a] | End of Tows[a] | | | | |
| G16b | 8/8/05 | south of southern tip of Harbor Island | 0.1 | 44 – 60 | 25 – 47 | 1 – 12 | 2 – 87 | 1.0 – 2.0 min | 1:3 – 1:6 | B1b | 2,300J |
| G17b | 8/9/05 and 8/10/05 | south of southern tip of Harbor Island | 0.1 | 23 – 37 | 18 – 38 | 6 – 16 | 5 – 61 | 1.0 – 1.5 min | 1:4 – 1:6 | DR002 | 320 |
| G18b | 8/10/05 | northwest of Duwamish/ Diagonal outfall | 0.3 | 33 – 44 | 30 – 46 | 2 – 18 | 11 – 62 | 0.75 – 1.5 min | 1:3 – 1:5 | K04 | 350 |
| G19b | 8/10/05 and 8/11/05 | east of Kellogg Island | 0.7 | 12 – 28 | 13 – 35 | 1 – 25 | 1 – 55 | 0.75 – 1.0 min | 1:2 – 1:5 | DR072 | 250 |
| G20b | 8/11/05 | mouth of Slip 1 | 0.9 | 23 – 35 | 21 – 30 | 5 – 82 | 32 – 35 | 0.5 – 0.75 min | 1:3 – 1:4 | DR021 | 170 |
| G21b | 8/9/05 | near west bank of waterway | 1.3 | 5 – 20 | 29 – 39 | 2 – 20 | 32 – 56 | 1 – 1.5 min | 1:3 – 1:14 | LDW-SS46 | 3,000 |

[a] Ten tows were performed at each sampling location.

[b] Depending on location, some tows were initiated at a distance from the target location and then towed towards the target location. Other tows were initiated on one side of the target location and towed through the location, ending on the other side of the location (see Figure 2-1). This latter approach occurred in Slip 1 because of limited space.

[c] The scope and/or the duration of the tow were changed during repeat tows if the sledge was empty or completely full of sediment when it was retrieved, or if the sledge caught on debris during the tow.

[d] Surface sediment sampling locations co-located with the gastropod target locations

[e] TBT concentration in a single grab or composite sediment sample previously collected, as reported by Windward (2005a, b, c)

dw – dry weight

After the shells were cracked, the gender of the meso- or neogastropods was determined, and the length of the male penises was measured before the males were returned to the sampling jar. The jars with the live specimens were stored in a cooler with ice. The females were examined for imposex using the methods 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[4] and penis. Table 2-2 presents the imposex stage criteria for this evaluation. 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.

**Table 2-2. Imposex stage criteria**

| IMPOSEX STAGE | CHARACTERISTICS |
|---|---|
| 1 | development of small penis or small section of vas deferens |
| 2 | development of either:<br>1) larger penis with a penis duct, or<br>2) two sections of vas deferens, or<br>3) both a penis and a vas deferens section |
| 3 | development of either:<br>1) larger penis with vas deferens section, or<br>2) a complete vas deferens, or<br>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 |

Source: Oehlmann et al. (1991)

Because the vas deferens could not be seen in imposexed females of *Nassarius mendicus*, penis length was measured in both genders to assess the level of imposex using the relative penis size (RPS) approach (Gibbs et al. 1988). The RPS index is calculated as follows:

RPS index = (mean length of female penis[3]/mean length of male penis[3]) x 100

## 3.0 Results

This section presents the results of gastropod field collection and the taxonomic identification and imposex analyses. Dr. Kohn's imposex report and laboratory data forms are presented in Appendix B. GPS coordinates of sampling locations are presented in Appendix A, and photocopies of field forms and notebooks are presented

[4] A sperm-carrying duct

in Appendix C. Copies of completed chain-of-custody forms used to track sample custody are presented in Appendix D.

## 3.1 GASTROPOD COLLECTION RESULTS

A total of 629 gastropods were collected at the six subtidal locations (Table 3-1), ranging from 21 specimens at location G21b to 190 specimens at location G20b. At two locations (G18b and G19b), more than 100 *A. gausapata* were collected. At these two stations, 100 individuals were retained, and the remaining specimens of *A. gausapata* were returned to the sampling location, as indicated in the methods memorandum (Windward 2004b).

**Table 3-1. Number of gastropods collected at each target location**

| GASTROPOD COLLECTION TARGET LOCATION ID | RIVER MILE | DEPTH START OF TOWS (ft MLLW) | DEPTH END OF TOWS (ft MLLW) | SUBSTRATE TYPE IN SLEDGE | TOTAL NUMBER OF GASTROPODS[a] |
|---|---|---|---|---|---|
| G16b | 0.1 | 44 – 60 | 25 – 47 | sand and debris | 98 |
| G17b | 0.1 | 23 – 37 | 18 – 38 | mud, shell fragments, debris, and rocks | 39 |
| G18b | 0.3 | 33 – 44 | 30 – 46 | mud and sand | 167 |
| G19b | 0.7 | 12 – 28 | 13 – 35 | mud, silt, and clay | 119 |
| G20b | 0.9 | 23 – 35 | 21 – 30 | mud, silt, clay, and fine sand | 190 |
| G21b | 1.3 | 5 – 20 | 29 – 39 | mud, shell fragments, debris, and rocks | 16 |

[a] The total numbers of gastropods counted in this field, as presented in this table, are different from those presented in Table 3-2 because the latter reflects gastropod counts made by Dr. Kohn in the laboratory. In addition, for a few samples, only a subset of total *A. gausapata* collected were counted.

## 3.2 TAXONOMIC IDENTIFICATION RESULTS

All gastropods were identified in the laboratory by Dr. Kohn prior to imposex analysis. The gastropods collected at the six locations were distributed over 12 taxa (species or genus) (Table 3-2). Of these 12 taxa, four were in the order Mesogastropoda, and four were in the order Neogastropoda. The most abundant species found at all locations was the neogastropod *A. gausapata* (formerly *Mitrella goldii*), which accounted for 45% of all gastropods identified by Dr. Kohn.[5] The next most common neogastropod, *O. baetica*, accounted for 5% of all the identified gastropods and *N. mendicus*, another neogastropod, accounted for 2% of all identified gastropods. Mesogastropods or other neogastropods were collected in low numbers (one to five individuals per taxon). Four other gastropod taxa from the orders Cephalaspidea and Pyramidellacea were collected during the study. The most

[5] Dr. Kohn discontinued identifying and counting *A. gausapata* from location G18b and G19b after 30 females had been examined for imposex, per consultation with EPA, because *A. gausapata* was highly abundant at these locations and imposex had not been observed in this species in either 2004 or 2005.

abundant of these gastropods, *Turbonilla* sp., was present throughout the study area and accounted for 40% of all gastropods identified.

**Table 3-2. Number of gastropod species identified and subjected to imposex analysis**

| GASTROPODS | | LOCATION ID | | | | | | TOTAL NUMBER OF GASTROPODS |
|---|---|---|---|---|---|---|---|---|
| TAXA | ORDER | G16b | G17b | G18b | G19b | G20b | G21b | |
| *Lacuna vincta* | Mesogastropoda | 0 | 0 | 2 | 0 | 0 | 0 | 2 |
| *Melanella* sp. | Mesogastropoda | 3 | 0 | 0 | 0 | 0 | 0 | 3 |
| *Natica* sp. | Mesogastropoda | 1 | 0 | 0 | 0 | 3 | 1 | 5 |
| *Polinices sp.* | Mesogastropoda | 0 | 0 | 4 | 0 | 1 | 0 | 5 |
| *A. gausapata* | Neogastropoda | 58[a] | 16 | 58[b] | 49[b] | 39 | 12 | 232 |
| *Kurtziella plumbea* | Neogastropoda | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| *N. mendicus* | Neogastropoda | 1 | 3 | 4 | 2 | 1 | 0 | 11 |
| *O. baetica* | Neogastropoda | 29 | 0 | 0 | 0 | 0 | 0 | 29 |
| *Acteocina* sp. cf *A. cerealis* | Cephalaspidea | 0 | 4 | 1 | 0 | 2 | 1 | 8 |
| *Acteon (Rictaxis) punctocaelata* | Cephalaspidea | 0 | 0 | 2 | 0 | 2 | 0 | 4 |
| *Odostomia* sp. | Pyramidellacea | 0 | 1 | 0 | 0 | 7 | 0 | 8 |
| *Turbonilla* spp. | Pyramidellacea | 8 | 5[c] | 54 | 17[c] | 120 | 3 | 207 |
| Total number of gastropods | | 101 | 29 | 125 | 68 | 175 | 17 | 515[d] |

[a] Five of the *A. gausapata* collected were found to be empty shells.

[b] One hundred specimens of *A. gausapata* from each of these locations were delivered to Dr. Kohn. The identification and imposex analyses were discontinued after 30 females of this species from each of these locations had been examined, as discussed with EPA.

[c] One of the *Turbonilla* sp. collected at G17b and one collected at G19b were found to be empty shells.

[d] Of the 629 gastropods collected in the field, Dr. Kohn identified and counted 515. The remaining gastropods were not counted because not all *A. gausapata* specimens were examined for imposex (see footnote b) and not all other gastropods (i.e., gastropods other than meso- and neogastropods) were identified and counted by Dr. Kohn.

## 3.3 IMPOSEX ANALYSIS RESULTS

This section presents the results of the imposex analysis conducted for meso- and neogastropods collected from the LDW. Gastropods in other orders were not examined for imposex because these specimens belong to genera that are normally hermaphroditic and therefore not subject to imposex.

### 3.3.1 Imposex analysis of Mesogastropoda

The order Mesogastropoda was represented by four taxa. Five specimens of *Polinices* sp. were examined, of which three were identified as immature males, one as an immature female, and one as a small female (0.7 cm) with no symptoms of imposex (Table 3-3). Five specimens of *Natica* sp. were examined; all individuals appeared to be immature with no evidence of imposex. One specimen of *Lacuna vincta* was identified

as a female with no symptoms of imposex. Another small specimen of *L. vincta* (0.5 cm) and three specimens of *Melanella* sp., the fourth mesogastropod, were not examined because they were too small (no length measurements were noted in the laboratory sheet).

**Table 3-3. Results of imposex analysis of mesogastropods collected in the LDW**

| LOCATION ID | *NATICA* SP | *POLINICES* SP | *LACUNA VINCTA* | *MELANELLA* SP | IMPOSEX ANALYSIS OF FEMALES |
|---|---|---|---|---|---|
| G16b | 1 immature specimen | nc | nc | 3 immature specimens | na |
| G17b | nc | nc | nc | nc | na |
| G18b | nc | 3 immature males and 1 female | 1 female and 1 immature specimen | nc | no symptoms of imposex |
| G19b | nc | nc | nc | nc | na |
| G20b | 3 immature specimens | 1 immature female | nc | nc | na |
| G21b | 1 immature specimen | nc | nc | nc | na |

nc – not collected; this species was not found at this location

na – not available; mature females were not collected at this location

### 3.3.2 Imposex analysis of Neogastropoda

The order Neogastropoda was represented by four taxa. The results of the imposex analysis for the three most abundant gastropods collected during the study, *A. gausapata, O. baetica*, and *N. mendicus*, are presented in Sections 3.3.2.1, 3.3.2.2, and 3.3.2.3, respectively. One specimen of *Kurtziella plumbea*, the fourth neogastropod, was not examined because it was too small (no length measurement was noted in the laboratory sheet).

#### *3.3.2.1 Astyris gausapata*

*A. gausapata* was collected at all locations, and individuals from each location were assessed for imposex by Dr. Kohn (Table 3-4). All specimens collected at G16b, G17b, G20b, and G21b were examined. In addition, 58 and 49 specimens out of 100 specimens collected at locations G18b and G19b, respectively, were examined. Dr. Kohn discontinued identifying and counting *A. gausapata* from locations G18b and G19b after 30 females from each location had been examined for imposex, per consultation with EPA, because *A. gausapata* was highly abundant at these locations and imposex had not been observed in this species in either 2004 or 2005. None of the females of this species that Dr. Kohn examined showed any symptoms of imposex.

**Table 3-4. Results of imposex analysis of *Astyris gausapata* collected in the LDW**

| Location ID | Number of Individuals Examined | Shell Lengths (cm) | Number of Females | Number of Males | Imposex Analysis of Females |
|---|---|---|---|---|---|
| G16b | 53 | 0.6-1.0 | 27 | 26 | no symptoms of imposex |
| G17b | 16 | 0.6-1.0 | 9 | 7 | no symptoms of imposex |
| G18b | 58 | 0.6-1.0 | 30 | 28 | no symptoms of imposex |
| G19b | 49 | 0.8-1.1 | 30 | 19 | no symptoms of imposex |
| G20b | 39[a] | 0.5-1.1 | 15 | 23 | no symptoms of imposex |
| G21b | 12 | 0.7-1.2 | 6 | 6 | no symptoms of imposex |

[a] One specimen was not examined because it was too small (0.3 cm).

#### *3.3.2.2 Olivella baetica*

*O. baetica* was found only at G16b where 29 specimens were collected. All specimens, except two very small specimens, were examined. Nineteen of the specimens were clearly females with no symptoms of imposex (Table 3-5). The shell lengths of the females ranged from 0.6 to 1.0 cm. Four specimens were considered to be males with a short penis (0.8 to 1.0 mm), dark yellow gonads, and no glandular oviducts. The shell lengths of the males ranged from 0.7 to 1.0 cm. The remaining four specimens were immature and were not assessed for imposex. The sex ratio based on gender determinations of the 23 specimens was biased towards females, and Dr. Kohn noted that it appears that females mature at a smaller size than males. However, because of the small sample size (gender determination of 23 specimens and size determination of four males), none of these findings is conclusive (see Appendix B for further details). The significance of these two observations is not known and the prevalence of the smaller maturation size of females is also unknown. Dr. Kohn noted that the largest observed specimens were only half of the typical adult size of 2 cm. The preponderance of smaller adults was only observed in *O. baetica*. The significance of this observation, and the effect it may have on imposex determination, is not known.

**Table 3-5. Results of imposex analysis of *Olivella baetica* collected in the LDW**

| Location ID | Number of Individuals Examined | Shell Lengths (cm) | Number of Females | Number of Males | Imposex Analysis of Females |
|---|---|---|---|---|---|
| G16b | 27[a] | 0.5 – 1.0 | 19 | 4 | no symptoms of imposex |

[a] Two of the 29 individuals of this species were not examined because they were too small (< 0.5 cm), and four of the 27 individuals examined were not evaluated because they were immature.

#### *3.3.2.3 Nassarius mendicus*

Eleven specimens of *N. mendicus* were collected. This species was found at all locations, except G21b (Table 3-6). Imposex analyses were performed on all five of the females collected. The degree of imposex in all female specimens of *N. mendicus* examined was similar to Stage 2 (see Table 2-2), except that in all cases penises were

present but no vasa deferentia[6] were observed. Stages 1 through 3 are described as early stages, with no impact on reproduction (Oehlmann et al. 1991).

### Table 3-6. Results of imposex analysis of *Nassarius mendicus*

| Location ID | Number of Individuals Examined | Shell Lengths (cm) | Number of Females | Number of Males | Imposex Analysis of Females |
|---|---|---|---|---|---|
| G16b | 1 | 1.4 | na | 1 | na |
| G17b | 3 | 1.3 – 1.6 | 1 | 2 | stage 2 imposex |
| G18b | 4 | 1.4 – 1.5 | 2 | 2 | both females stage 2 imposex |
| G19b | 2 | 1.2 and 1.5 | 2 | na | both females stage 2 imposex |
| G20b | 1 | 1.6 | na | 1 | na |
| G21b | nc | na | na | na | na |

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

nc – not collected; species was not found at this location

RPS indices for each of the locations where female *N. mendicus* were collected are presented in Table 3-7, based on the data presented in Table 3-8. All RPS indices were 2.6% or less. Spence et al. (1990) states that, in general, sterile females are absent at RPS indices below 5%. The percentage of sterility increases when the RPS index is between 5% and 40%, and at RPS indices exceeding 40%, most or all females are sterile.

### Table 3-7. RPS indices for *Nassarius mendicus* collected from the LDW

| Location ID | Mean Female Penis Length (mm) (±stdev) (number measured) | Mean Male Penis Length (mm) (±stdev) (number measured)[a] | RPS Index[b] |
|---|---|---|---|
| G17b | 3.3 (1) | 11.2 ± 1.5 (5) | 2.6 |
| G18b | 3.1 ± 0.9 (2) | 11.2 ± 1.5 (5) | 2.1 |
| G19b | 2.6 ± 0.1 (2) | 11.2 ± 1.5 (5) | 1.2 |

[a] Penis length in males is not expected to be affected by TBT concentrations, so the RPS index at each location was calculated using the mean penis length from all locations.

[b] Dr. Kohn calculated a RPS index of 1.8 by using the mean female penis length and the mean male penis length from all stations (Appendix B). Dr. Kohn also calculated station-specific RPS indices for the two stations that had males and at least one female with imposex. The RPS indices calculated by Dr. Kohn for stations G17b and G18b were 2.0 and 3.4, respectively.

### Table 3-8. *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) |
|---|---|---|---|---|
| G16b | na | na | nm | Specimen 1 – 1.4 |
| G17b | Specimen 1 – 3.3 | Specimen 1 – 1.3 | Specimen 1 – 12.5<br>Specimen 2 – 11.7 | Specimen 1 – 1.6<br>Specimen 2 – 1.5 |
| G18b | Specimen 1 – 2.5<br>Specimen 2 – 3.7 | Specimen 1 – 1.5<br>Specimen 2 – 1.4 | Specimen 1 – 10.0<br>Specimen 2 – 9.1 | Specimen 1 – 1.5<br>Specimen 2 – 1.4 |

[6] Plural of vas deferens

| Location ID | Female Penis Length (mm) | Female Shell Height (cm) | Male Penis Length (mm) | Male Shell Height (cm) |
|---|---|---|---|---|
| G19b | Specimen 1 – 2.5<br>Specimen 2 – 2.6 | Specimen 1 – 1.2<br>Specimen 2 – 1.5 | na | na |
| G20b | na | na | Specimen 1 – 12.5 | Specimen 1 – 1.6 |

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

nm – not measured because no females of this species were collected at this location

## 4.0 Summary

The results of the imposex analysis are summarized in Table 4-1. In summary, imposex was not observed in any of the mesogastropods collected or in two of the three neogastropod species examined. Imposex was not observed in the neogastropods *A. gausapata* or *O. baetica*, including specimens collected from areas within the LDW that historically have had the highest TBT concentrations in sediment. The absence of imposex in *A. gausapata* in 2005 is consistent with the results of the 2004 pilot survey (Windward 2004c). *O. baetica* was not evaluated for imposex in the 2004 pilot survey.

*N. mendicus* was the only gastropod species collected that showed imposex symptoms. This is consistent with the results from the 2004 pilot survey (Windward 2004c). All females collected were affected, and all were classified at Stage 2, specifically Stage 2a (large penis with penis duct) following the scheme of Oehlmann et al. (1991). Penises were present in all, but none had an evident vas deferens. The calculated RPS indices for females collected in 2005 ranged from 1.2 to 2.6%, which is similar to the range (0.2 to 1.8%) observed for females of the same species collected in 2004. These RPS indices are all below thresholds associated with sterility in female neogastropods (Spence et al. 1990). Furthermore, in all other gastropods that have been studied in other locations, Stage 2 imposex has not interfered with female reproduction (Gibbs and Bryan 1996; Spence et al. 1990). It is thus probable that the level of imposex observed in *N. mendicus* in the LDW does not have an effect on reproduction.

Low numbers of *N. mendicus* (1 to 4) were collected at five out of the six sampling locations, and females were collected at three of the six locations. No *N. mendicus* females were collected at the two locations with the highest TBT concentrations or the location with the lowest TBT concentration. The uncertainty associated with these and other findings (e.g., the percentage of immature individuals on the imposex evaluation) will be discussed in the Phase 2 ERA.

## Table 4-1. Summary of results from the 2005 imposex study

| Location ID | TBT Sed Conc (µg/kg dw)[a] | Imposex Analysis Results | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mesogastropods | | Neogastropods | | | | | | |
| | | | | *A. gausapata* | | *O. baetica* | | *N. mendicus* | | |
| | | Imposex Symptoms? | # Females Examined | Imposex Symptoms? | # Females Examined | Imposex Symptoms? | # Females Examined | Imposex Symptoms? | # Females Examined | RPS Index |
| G16b | 2,300J | na | na | none | 27 | none | 19 | na | na | na |
| G17b | 320 | na | na | none | 9 | nc | nc | stage 2 | 1 | 2.6 |
| G18b | 350 | none | 2 | none | 30 | nc | nc | stage 2 | 2 | 2.1 |
| G19b | 250 | na | na | none | 30 | nc | nc | stage 2 | 2 | 1.2 |
| G20b | 170 | na | na | none | 15 | nc | nc | na | na | na |
| G21b | 3,000 | na | na | none | 6 | nc | nc | na | na | na |

[a] TBT concentration in a single grab or composite sediment sample previously collected, as reported by Windward (Windward 2005a, b, c)

dw – dry weight

na – not available; mature females of this species were not collected at this location, so imposex analyses could not be conducted

nc – not collected; species was not found at this location

## 5.0 References


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## Oversize Figures

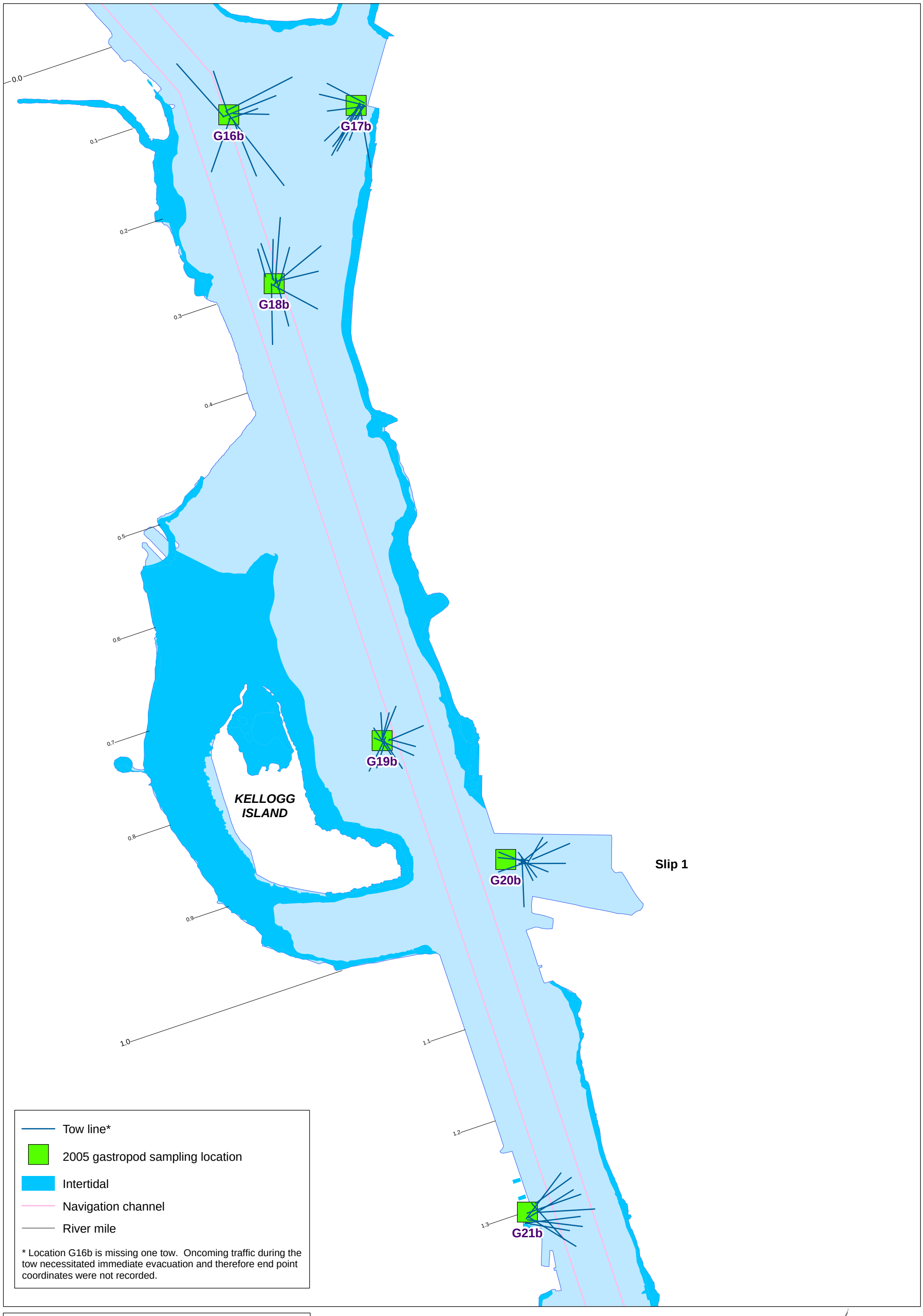


Figure 2-1. 2005 Gastropod tow lines of benthic sledge based on boat position

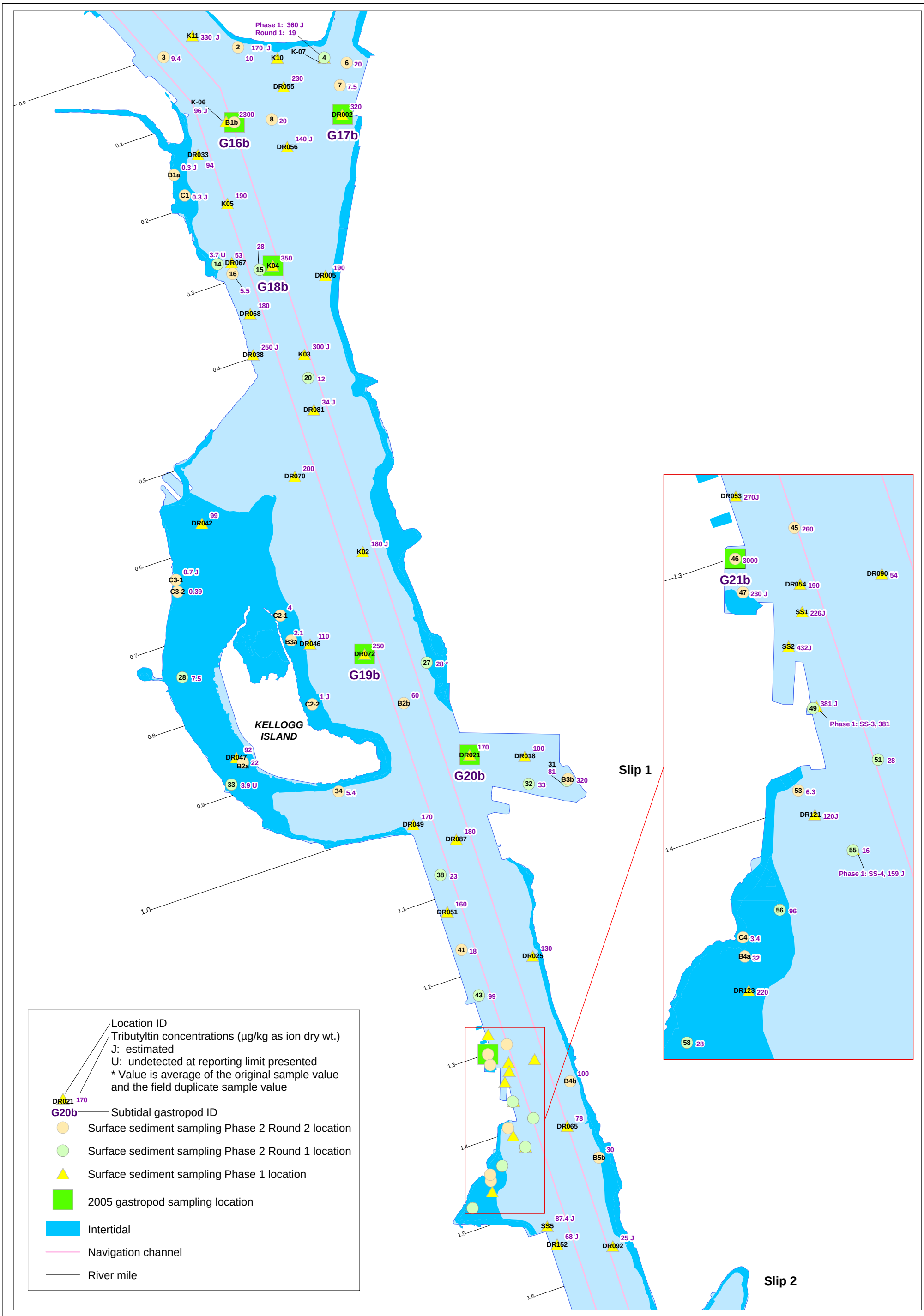


Figure 2-2. Gastropod imposex sampling locations and Phases 1 and 2 TBT concentrations in surface sediment samples (RM 0.0-1.6)

Prepared by STS 02/08/06 Map 1814

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