King County Brightwater TREATMENT SYSTEM

Geoduck Tissue Physical Characteristics, Bacteria, and Chemistry

Final Report

November 2002

King County
Department of Natural Resources and Parks
Wastewater Treatment Division

Brightwater Treatment System

Geoduck Tissue Study for the Brightwater Marine Outfall

November 2002

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Executive Summary

King County conducted two phases of site evaluation in 2000 and 2001 for the marine outfall for the Brightwater Regional Wastewater Treatment System. During these selection processes, the King County Council-adopted policy siting criteria were used to identify potentially suitable locations along the shoreline of northern King and southern Snohomish Counties for the outfall and diffuser. At the conclusion of the second phase of the outfall zone-narrowing process, the King County Council accepted candidate marine outfall Zones 5, 6, 7N, and 7S for further analysis and review in an Environmental Impact Statement for the Brightwater Regional Wastewater Treatment System (King County Ordinance 14278, Dec. 13, 2001).

The present-phase work further analyzes the characteristics of the candidate outfall zones. King County collected and analyzed geoduck (*Panopea abrupta*) tissue samples from three of the four candidate outfall zones (Zone 5 was dropped from consideration) to evaluate physical, bacterial, and chemical characteristics of this long-lived, sessile organism. The shells were also analyzed to determine geoduck ages. This work was performed in conjunction with a comprehensive geoduck population survey in the outfall study area.

Twenty-seven geoducks were collected in April and May 2002 for analysis of physical characteristics (age, weight, lipid concentration) and bacterial, trace metal, and trace organic parameters. Nine whole-body samples were analyzed for indicator bacteria. Nine whole-body and nine edible-portion samples were analyzed for physical characteristics, along with trace metals and trace organics. Edible-portion samples had the visceral ball and siphon skin removed prior to processing and analysis. The age of all 27 geoducks was determined to evaluate possible correlations between tissue-chemical concentrations and organism age.

Sample collection and analytical methodologies followed accepted regional protocols to provide data that would allow comparison to data from previous and future studies in Puget Sound.

Evaluation of geoduck shell-aging data indicates that these organisms are long-lived, with an age range of 9 to 95 years recorded for the 27 geoducks collected for this study. Bacterial counts were similar to other types of shellfish collected from relatively unimpacted areas in other King County studies and were below US Food and Drug Administration “safety level” guidance criteria.

Tissue concentrations of arsenic, cadmium, chromium, mercury, and nickel were all well-below FDA guidance or regulatory criteria. Mercury concentrations were found to increase with increasing geoduck age. Lead detected in eight of nine whole-body samples exceeded the FDA guidance criterion by as much as six times. The lead appears to be concentrated in the visceral ball, which contains most of the geoduck’s internal organs.

Six organic compounds were detected in geoduck tissue. One of these compounds, benzoic acid, is naturally occurring and another, bis(2-ethylhexyl) phthalate, may have been imparted to samples during tissue preparation and processing. Three isomers of the pesticide BHC (benzenehexachloride) were detected in one or more of the samples. These pesticides have not been detected in other shellfish samples and were not found in sediment samples recently collected from the geoduck study area.

1.0. INTRODUCTION

The Metropolitan King County Council approved the Regional Wastewater Services Plan (RWSP) in November 1999 (King County Ordinance 13680, Nov. 23, 1999). The RWSP called for a significant upgrade to King County's existing wastewater system including the construction of a new regional wastewater treatment plant in northern King or southern Snohomish County with a marine outfall to discharge treated effluent to Puget Sound. Using King County Council-adopted policy siting criteria, the County conducted two phases of outfall site evaluation in 2000 and 2001. These policy siting criteria were used to identify suitable locations along the shoreline of northern King and southern Snohomish Counties for the outfall and diffuser. At the conclusion of the second phase of outfall site evaluation, the King County Council accepted candidate marine outfall zones 5, 6, 7N, and 7S for further analysis and review in an Environmental Impact Statement (EIS) for the Brightwater Regional Wastewater Treatment System (King County Ordinance 14278, Dec. 13, 2001).

This report presents results of a baseline bacterial and chemical characterization study of geoduck (Panopea abrupta) tissue collected from three of the four candidate marine outfall zones identified in Phase 2; Zones 6, 7N, and 7S (during the first step of the Phase 3 site-screening process, Zone 5 was dropped from consideration). The study was performed as part of King County's Marine Outfall Siting Study (MOSS), which is evaluating potential locations for the new outfall that will discharge secondary-treated wastewater to Puget Sound. The report includes a project description, a summary of sampling and analytical methodologies used during the study, analytical results, and conclusions. Data summary tables referenced in this report are included in Appendix A.

1.1 Project Background

The purpose of this study was to perform a baseline bacterial and chemical characterization of geoduck tissue collected from the nearshore environment within the candidate marine outfall zones for King County's planned Brightwater Regional Wastewater Treatment System. The tissue study was conducted as part of a larger geoduck distribution and abundance survey (Golder 2002). Information from these studies may be used to evaluate potential impacts from operation of the marine outfall. Any potential impacts will be addressed in the Brightwater EIS.

The primary goals of the MOSS geoduck tissue study are to establish baselines within the candidate marine outfall zones for chemical body burden and indicator bacteria tissue concentrations. These geoduck tissue baselines may be used as benchmarks against which results from any possible future monitoring during outfall operation might be compared.

1.2 Site Description

The candidate marine outfall zones from which geoducks were collected are located offshore of southwest Snohomish County and northwest King County (Figure 1). Two of the candidate outfall zones are located near Edwards Point, and one is located near Point Wells. Geoducks were collected at depths ranging between 17 and 65 feet referenced to mean lower low water (MLLW).

Bathymetry at these depths ranges from "gently sloping" between Point Wells and Edwards Point to "steeply sloping" near each of the Points. Sparse to dense eel grass is present in all three zones from which geoducks were collected (Battelle 2001). Bottom substrates in the three collection areas have been classified as:

- a steep sand and gravel slope and sandy flat in Zone 6;
- sandy in Zone 7N; and
- fine sand with shell debris in Zone 7S (Golder 2002).

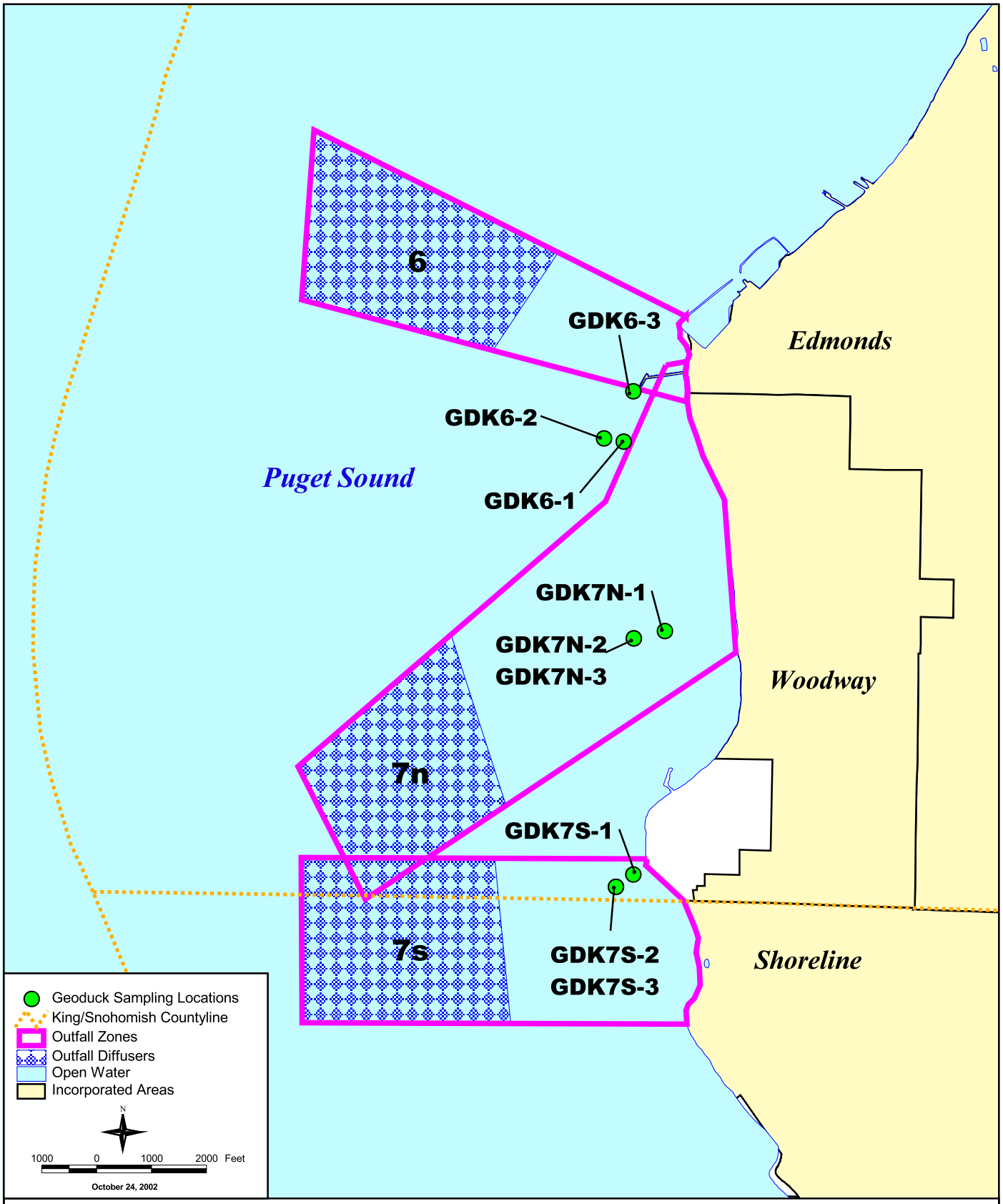

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Figure 1

Geoduck Tissue Sampling Locations

2.0. SAMPLE COLLECTION

Geoducks were collected for this tissue study as part of a larger survey performed to assess geoduck distribution and abundance in candidate marine outfall zones and the surrounding area (Golder 2002). Twenty-seven geoducks were collected in April and May 2002 for analysis of bacterial and chemical parameters, as well as measurement of age, weight, and lipid concentration. Sampling procedures may be found in the project sampling and analysis plan (King County 2002a)

2.1 Sampling Locations

Sampling stations were selected to provide areal coverage of three candidate marine outfall zones, with geoducks collected from similar depths in each zone. Sampling stations were also located in areas at which sediment chemistry data had recently been collected.

Geoduck tissue samples were collected at three locations in the MOSS study area; Zone 6, Zone 7N, and Zone 7S (Figure 1). A transect, set perpendicular to the shoreline, was completed at each of the three locations as part of the larger geoduck survey. Geoducks were collected from planned depths of approximately 20, 45, and 70 feet MLLW at each of the transects (actual sampling depths are included in Table A-1, Appendix A). Three geoducks were collected at each sampling location. Two animals were collected for physical and chemical analysis; one animal was analyzed as a "whole-body" sample, while only the "edible-portion" from the second animal was analyzed. The third geoduck was used for bacterial analysis as a whole-body sample.

Table A-1 (Appendix A) provides the coordinates for the nine geoduck tissue sampling stations along with actual sampling depths and associated candidate outfall zone designations. Two "locator" names were assigned to each sampling station to distinguish samples for which tissue from the whole animal (W) was analyzed and for which only the edible portion (E) was analyzed.

2.2 Sample Collection, Handling, and Processing

Geoducks were collected according to procedures summarized in Study Plan for a Survey of Geoduck Clams and Other Marine Biological Resources for the King County MOSS Project in the Point Wells-to-Edmonds Area (Appendix A of King County 2002a). Sample collection procedures followed protocols recommended by the Washington State Department of Fish and Wildlife with some exceptions, which are noted in the study plan.

Geoducks were collected by SCUBA divers who, after locating the geoduck's siphon, used a water jet to expose the siphon and remove the animal from the sediment. Geoducks were stored in mesh bags hanging from the dive vessel until they were transferred to ice-filled coolers at the dock. Effort was made to minimize contamination to the geoducks, however, these animals are not able to completely contract their tissue

into their shells. Some geoduck tissue came into contact with divers' neoprene gloves and the mesh storage bags. The geoducks were to be stored in individual, acid-washed, glass jars, however, all of the animals were too large and were instead stored in individual, Ziploc®-type bags. Geoducks were delivered to the King County Environmental Laboratory on the day of collection for tissue processing and sample preparation.

The initial preparation of all samples included appropriate cleaning and shucking in a manner that minimized contamination. Geoducks designated for bacterial analysis were segregated and shucked aseptically, according to US Food and Drug Administration (FDA)-recommended protocol (APHA 1985). Geoducks designated for chemical analysis were shucked in a manner that minimized potential contamination by metals and organic compounds (King County 2002a). All sample preparation was performed in a manner that preserved the integrity of the geoduck shells to facilitate aging of the organisms.

Shucked, whole-body samples for bacterial analysis were homogenized in individual, sterilized, stainless-steel blender jars outfitted with stainless-steel blades. Bacterial analysis was performed immediately following sample preparation. Edible-portion samples for chemical analysis were further prepared by removing the outer skin and visceral ball in a manner that minimized potential contamination by metals or organic compounds. Whole-body and edible-portion samples for chemical analysis were homogenized in a blender outfitted with a titanium blade and individual, acid-cleaned, glass blender jars. Equipment rinsate blanks were collected both prior to and after processing of tissue samples for analysis of trace metals in order to assess potential cross-contamination between samples.

3.0. SAMPLE ANALYSIS

A brief summary of the analytical parameters and associated methodologies for physical, bacterial, and chemical analyses used during this study is presented in the following section. Complete documentation of project analytical methodologies may be found in the project sampling and analysis plan (King County 2002a). The complete list of analytical parameters is shown in the data report included in Appendix B.

The King County Environmental Laboratory performed all physical, bacterial, and chemical analyses with the exception of organism aging, which was performed by Brent Vadopalas and staff of the Marine Molecular Biotechnology Lab at the University of Washington School of Aquatic and Fishery Sciences.

Chlorinated herbicides were originally included in the suite of analytes. Analysis of these compounds was subcontracted to Severn Trent Laboratories, Inc. (STL), an analytical laboratory in Tacoma, Washington. STL experienced serious analytical difficulties with the geoduck samples and was unable to provide any sample results. A summary of the analytical difficulties is provided as part of the quality control narrative included in Appendix C.

3.1 Physical Analysis

Physical parameters analyzed included total solids, percent lipids, weight, and organism age. Total solids analysis was performed according to Standard Method (SM) 2540G, a gravimetric determination of percent solids (APHA, 1998). Percent lipid analysis was performed according to King County Environmental Laboratory analytical methodology (King County 1998), which employs a gravimetric determination of the percent lipid in a sample subsequent to extraction with methylene chloride and acetone.

Age estimates for geoducks were obtained using a 5-step process including: cutting of the hinge plates; thin sectioning of the shell; mounting of thin sections; polishing slides; and estimating specimen age. A 4 x 5 centimeter section containing the hinge plate was removed from the right valve using a small circular saw. A minimum of three 2-millimeter thin sections were taken from each hinge plate using a slow speed diamond saw. The sections were slide-mounted and polished using 800 grit sandpaper. Transmitted light microscopy was utilized to visualize annual growth rings. The growth rings revealed year-specific width variation, regionally consistent across samples. These patterns may correlate with localized environmental factors or regional oceanographic conditions and were utilized to establish year-reference points in series of growth rings. At least three independent estimates were made for each specimen. Reported ages are +/- 2 years for ages less than 30, and +/- 5 years for ages greater than 30.

3.2 Bacterial Analysis

Bacteria analyzed in the whole-body tissue samples included fecal coliforms, enterococci, and *Escherichia coli*. Analysis of fecal coliform, enterococcus, and *E. coli* bacteria were performed according to the Multiple Tube Fermentation method and the Most Probable Number (MPN) method of concentration estimation (APHA 1985 and 1995 and FDA 1998, respectively).

3.3 Trace Metals Analysis

Trace metals analyzed in both whole-body and edible-portion tissue samples included antimony, arsenic, beryllium, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, thallium, tin, and zinc. Mercury was analyzed by cold vapor atomic absorption spectroscopy (CVAA). Analysis of all other elements was initially conducted by inductively coupled plasma optical emission spectroscopy (ICP-OES). Those elements for which ICP-OES results were less than the method detection limit (MDL) were reanalyzed by inductively coupled plasma mass spectroscopy (ICP-MS) to achieve a lower detection limit. All trace metals analyses were performed according to methodology specified under the Puget Sound Estuary Program (PSEP 1996).

3.4 Trace Organics Analysis

Trace organic compounds analyzed in both whole-body and edible-portion tissue samples included base/neutral/acid extractable semivolatile (BNA) compounds, butyltin isomers, chlorinated pesticides and polychlorinated biphenyls (PCBs), chlorinated herbicides, and organophosphorus pesticides. BNA analysis was performed according to EPA Method 3550/8270 (SW 846), which employs solvent extraction with sonication and analysis by gas chromatography/mass spectroscopy (GC/MS). Butyltin analysis was performed according to a National Oceanic and Atmospheric Administration (NOAA) method (Krone et al.1989), which employs a methylene chloride extraction with tumbling followed by derivitization through a Gringnard reaction and analysis by GC/MS in the Selected Ion Mode (SIM). Chlorinated Pesticide/PCB analysis was performed according to EPA method 3550/8081/8082 (SW 846), which employ solvent extraction with sonication and analysis by gas chromatography/electron capture detector (GC/ECD) with dual column confirmation. Organophosphorus pesticide analysis was performed according to EPA Method 3540C/8141A (SW 846), which employs solvent extraction with soxhlet and analysis by GC/MS (SIM). Analysis of chlorinated herbicides was attempted using EPA Method 8151A Modified (SW-846). A summary of the analytical difficulties experienced during chlorinated herbicide analysis in included in Appendix C.

3.5 Laboratory Quality Control

The quality control (QC) samples analyzed in association with geoduck tissue physical and chemical parameters are summarized in Table 1. The analytical frequency for all physical and chemical QC samples except surrogates was 1 per QC batch (20 samples

maximum). Surrogates were analyzed with every sample for all organic analyses except percent lipids.

Table 1
Quality Control Samples for Geoduck Tissue Chemistry Analysis

Analysis	Method Blank	Spike Blank	Lab Replicate	Matrix Spike	Reference Material	Surrogates
Total Solids	Yes	No	Yes	No	No	No
Percent Lipids	Yes	No	Yes	No	No	No
Metals	Yes	Yes	Yes	Yes	Yes	No
BNAs	Yes	Yes	Yes	Yes	Yes	Yes
Butyltin Isomers	Yes	Yes	Yes	Yes	No	Yes
OP Pesticides	Yes	Yes	Yes	Yes	No	Yes
Cl. Pest./PCB	Yes	Yes	Yes	Yes	Yes	Yes

One additional set of QC samples was analyzed for trace metals. Pre- and post-homogenization rinsate blanks were collected from the tissue processing equipment and analyzed to assess cross-contamination between samples.

Routine bacterial QC analysis monitored the method performance of each sample analysis batch for each of the three methods. Bacterial QC samples included lab duplicates, negative controls, positive controls, and sterility controls.

All geoduck tissue study QC results are located in Appendix C, which includes a narrative summary of QC issues as well as all QC data.

4.0. ANALYTICAL RESULTS AND DISCUSSION

This section presents a summary of results from physical, bacterial, and chemical analyses performed on 27 geoduck tissue samples. Complete analytical results including method detection limits (MDLs) are presented in Appendix B. Analytical results for physical and microbiological parameters and detected chemicals are summarized in Tables A-2 through A-5 (Appendix A). All data discussed in this section are presented on a wet-weight basis.

4.1 Physical Characteristics

Physical characteristics, including age, weight, percent solids, and percent lipids, are summarized in Table A-2 (Appendix A).

Geoduck ages ranged from 9 to 95 years with mean and median values of 50 and 54 years, respectively. Geoducks can be exceptionally long-lived; the record individual animal has been aged to 168 years (Underwater Harvesters Association 2002).

Geoduck weights (whole body plus shell) ranged from 0.55 to 1.95 kilograms (Kg) with mean and median values of 1.15 and 1.16 Kg, respectively. A value of 1.06 Kg was reported as the mean weight of 110 geoducks collected for biomass measurement during the larger geoduck survey (Golder 2002).

Percent lipid results ranged from 0.11 to 0.74% with mean and median values of 0.30 and 0.24%, respectively. Lipid values in other bivalve species evaluated by King County include: 0.49% for butter clams (*Saxidomus giganteus)*, which is a 3-year mean value for 1999 to 2001 (King County 2001, 2002b); and 1.31% for mussels *(Mytilus galloprovinciallis)*, which is a 2-season mean value for autumn 1996 and spring 1997 (King County 1999).

4.2 Bacteria

Bacterial results are summarized in Table A-3 (Appendix A). Fecal coliform bacteria were detected in 6 of 9 whole-body samples at counts ranging from 20 to 310 MPN/100g (most probable number per 100 grams). *Escherichia coli* bacteria were detected in 7 of 9 whole-body samples at the same range of counts as fecal coliform bacteria. The MDL for these bacterial analyses is 20 MPN/100g. Enterococcus bacteria were detected in all 9 whole-body samples at counts ranging from 20 to 220 MPN/100g

E. coli results were very similar to fecal coliform results, however, enterococcus results were different than both fecal coliform and *E. coli* results. These similarities and differences are expected as *E. coli* and fecal coliform bacteria belong to the same bacterial group (coliforms) while enterococcus bacteria belong to a separate bacterial group (fecal streptococci). The range of bacterial counts detected in these geoduck

samples is similar to bacterial counts detected in butter clams collected from relatively unimpacted areas as part of an ongoing King County monitoring program (King County 2001).

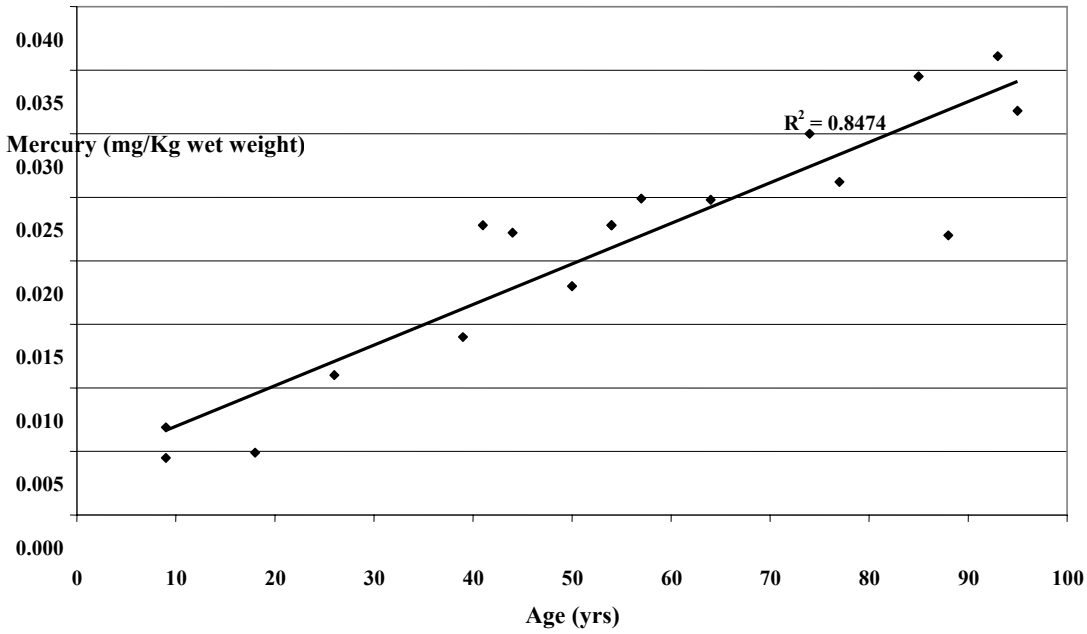
The State of Washington does not have regulatory or guidance criteria for bacterial levels in shellfish tissue. Federal guidance (FDA 2001) suggests the following **safety levels** for *E. coli* or fecal coliform tissue concentrations in commercially-traded domestic clams: "*1 or more of 5 subsamples exceeding MPN of 330/100 grams or 2 or more exceeding 230/100 grams.*" Bacterial levels for both fecal coliform and *E. coli* in the 9 whole-body geoduck tissue samples met these federal guidance criteria.

4.3 Trace Metals

Fourteen metals were analyzed in geoduck tissue samples. Analytical results for detected metals are summarized in Table A-4 (Appendix A). Complete analytical results, including MDLs, are presented in Appendix B.
Eleven metals were detected in all 18 tissue chemistry samples; arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, tin, and zinc. Antimony was detected in 8 of 9 whole-body samples but was not detected in edible-portion samples. Beryllium and thallium were not detected in any samples.

There is no apparent correlation between geoduck age and trace metals tissue concentrations with the exception of mercury. Figure 2 shows a possible correlation between organism age and increasing mercury tissue concentration. The R^2 value is 0.8474, based on a sample size of 18, combining both whole-body and edible-portion samples.

Figure 2
Geoduck Tissue Mercury Concentration Compared to Age



4.3.1 Geoduck Tissue Metal Concentrations Compared to FDA Guidance Values

The FDA has established guidance values called **Levels of Concern** (LOC) for five metals in shellfish tissue; arsenic, cadmium, chromium, lead, and nickel (FDA 1993a, b, c, d, e). Table 5 compares geoduck tissue concentrations from this study and associated LOC values. These comparisons are based on wet weight concentrations of the five metals.

4.3.2 Geoduck Tissue Concentrations (mg/Kg wet weight) Compared to FDA Levels of Concern

Table 2
(n = 9 for Both Whole-Body and Edible-Portion Samples)

Metal	Whole			Edible			LOC
	Min	Mean	Max	Min	Mean	Max	
Arsenic	1.9	3.71	6.89	1.4	2.4	3.74	55
Cadmium	0.214	0.294	0.452	0.045	0.12	0.226	3
Chromium	0.272	0.405	0.622	0.10	0.18	0.303	11
Lead	0.201	2.62	4.71	0.014	0.0527	0.291	0.8
Nickel	0.313	0.720	1.30	0.0786	0.221	0.640	80

- In general, concentrations of these five metals are lower in edible-portion samples than in corresponding whole-body samples, which included the siphon skin and visceral ball. Lead concentrations, in particular, were much higher in whole-body samples; between 9 and 277 times the concentrations detected in corresponding edible-portion samples.
- Arsenic, cadmium, chromium, and nickel concentrations in both edible-portion and whole-body samples are all below LOC values.
- Lead concentrations in edible-portion samples are all below the LOC value. Lead concentrations in 8 of 9 whole-body samples exceed the LOC value; the maximum lead concentration is almost 6 times greater than the LOC value.

The FDA has also established a regulatory **Action Level** of 1.0 mg/Kg for mercury in fish and shellfish tissue (FDA, 1995). Food products that exceed an Action Level cannot be commercially traded; an important distinction from the non-regulatory Levels of Concern. Geoduck tissue mercury concentrations range from 0.0045 to 0.0361 mg/Kg. The highest geoduck tissue mercury concentration detected in this study is approximately 28 times lower than the FDA Action Level.

4.3.3 Geoduck Tissue Metal Concentrations Compared to Other Shellfish Tissue

King County has analyzed butter clam (*Saxidomus giganteus*) tissue for trace metals on an annual basis since 1998 (King County 2000, 2001, and 2002b). The County also analyzed mussel (*Mytilus galloprovinciallis*) tissue for trace metals in a two-season study performed in October 1996 and April 1997 (King County 1999). Butter clams are collected from Puget Sound beaches in an area extending from Richmond Beach to Normandy Park. The mussel study included both transplanted and ambient mussels in the Duwamish River and Elliott Bay, as well as control mussels from Totten Inlet. The following table provides a comparison between these three types of bivalves of the minimum, mean, and maximum values for the following metals; arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, and zinc. For comparison purposes, the concentrations detected in whole-body geoduck tissue samples have been used in order to be comparable with the butter clam and mussel data, which are also from whole-body samples.

Table 3
Trace Metal Concentrations in Geoducks, Butter Clams, and Mussels (mg/Kg wet weight)

Metal	Geoducks (n = 9)			Butter Clams (n =26)			Mussels (n =82)		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
Arsenic	1.90	3.71	6.89	2.31	3.13	4.48	0.34	0.83	1.85
Cadmium	0.214	0.294	0.452	0.043	0.060	0.097	0.189	0.415	0.840
Chromium	0.272	0.405	0.622	0.273	0.464	0.822	0.059	0.145	0.934
Copper	5.01	6.85	12.6	1.14	1.92	4.05	0.51	1.14	2.18
Lead	0.201	2.62	4.71	0.041	0.125	0.265	0.024	0.245	0.833
Mercury	0.011	0.024	0.036	0.005	0.009	0.014	0.004	0.009	0.023
Nickel	0.313	0.720	1.30	0.664	0.911	1.31	0.051	0.153	0.420
Selenium	1.00	1.49	2.00	0.20	0.39	0.61	N/A	N/A	N/A
Silver	1.19	4.55	8.20	0.18	0.79	1.45	0.013	0.022	0.032
Zinc	11.6	19.9	61.3	11.3	14.3	17.6	4.8	18.0	49.1

4.4 Trace Organics

Six organic compounds out of 109 compounds analyzed were detected in one or more tissue chemistry samples. Analytical results for the six detected compounds are summarized in Table A-5 (Appendix A). Complete analytical results, including MDLs, for all 109 organic compounds are presented in Appendix B.

4.4.1 Alpha-BHC, Beta-BHC, and Gamma-BHC (Lindane)

Alpha-BHC was detected in all 18 tissue chemistry samples at concentrations ranging from 1.8 to 15.3 µg/Kg. Beta-BHC was detected in 11 of 18 samples at concentrations ranging from 1.8 to 7.19 µg/Kg. Gamma-BHC, also known by the trade name Lindane, was detected in 2 of 18 samples at concentrations of 1.4 and 1.6 µg/Kg. The MDL for these three compounds was 1.3 µg/Kg. There was no apparent correlation between alpha- or beta-BHC concentrations and geoduck age, lipid concentration, or depth from which the organism was collected.

BHC (benzenehexachloride) is an organochlorine pesticide that exists as eight isomers. Technical-grade BHC (the most common insecticide form) is a mixture of five isomers; alpha, beta, delta, epsilon, and gamma (Lindane). Technical-grade BHC has been banned in the United States due to concerns over its potential as a human carcinogen, teratogen, and mutagen and has not been produced in the United States since 1983. Although Lindane has not been produced in the United States since 1977, it is still imported and licensed for use as a prescription remedy for head lice and in veterinary shampoos, as well as an insecticide and acaricide on many crops. BHC, which is fat-soluble, is considered a persistent, bioaccumulative compound that can magnify through the food chain (California Air Resources Board 2002, Cornell University 2002, EPA 2002).

BHC compounds were not detected in nearshore sediment samples collected in December 2001 within the geoduck study area (King County 2002c). BHC compounds have not been detected in other shellfish samples collected by King County (King County 2000, 2001, 2002b).

4.4.2 Bis(2-ethylhexyl) Phthalate

Bis(2-ethylhexyl) phthalate was detected in 16 of 18 tissue chemistry samples at concentrations ranging from 48 to 445 µg/Kg. The MDL for this compound was 32 µg/Kg. There is no apparent correlation between reported bis(2-ethylhexyl) phthalate tissue concentrations and geoduck age, lipid concentration, or depth from which the organism was collected.

Bis(2-ethylhexyl) phthalate is a common plasticizer found in many products such as polyvinyl chloride (PVC), rubber, cellulose, and styrene (EPA 2002b). Geoducks were stored in plastic, Ziploc®-type storage bags between sample collection and tissue processing as well as being exposed to divers' neoprene gloves and mesh bags. Laboratory personnel also wore plastic surgical gloves during tissue dissection and homogenization. Although bis(2-ethylhexyl) phthalate was not detected in the laboratory method blank associated with geoduck tissue analysis, the quality control review narrative (Appendix C) indicates that storage and handling issues mentioned above may have imparted trace amounts of this compound to the tissue samples. Edible-portion samples, which were handled more extensively during processing, have consistently higher reported bis(2-ethylhexyl) phthalate concentrations than the corresponding whole-body samples. It appears, therefore, that bis(2-ethylhexyl) phthalate concentrations detected in geoduck tissue samples are likely artifacts of contamination imparted during sample collection and processing.

Low concentrations of bis(2-ethylhexyl) phthalate were detected in sediment samples collected in December 2001 within the geoduck study area (King County 2002c). These concentrations ranged from 2.5 to 8.5 mg/Kg normalized to organic carbon (9.5 to 93 µg/Kg on a dry-weight basis).

4.4.3 Benzoic Acid

Benzoic acid was detected in 17 of 18 tissue chemistry samples at concentrations ranging from 230 to 26,000 µg/Kg. The MDL for this compound is 210 µg/Kg. The benzoic acid concentration detected in each whole-body sample is between 3 and 56 times higher than the concentration detected in the corresponding edible-portion sample. There is no apparent correlation between geoduck age and benzoic acid concentration.

While benzoic acid is widely used as a food preservative, in tobacco processing, and as a dye enhancer (among many uses), it is also a naturally-occurring compound that is prevalent in shellfish as a degradation product of metabolic processes (DOH 1996). The higher benzoic acid concentrations detected in the whole-body tissue samples in which the visceral ball was kept intact can, most likely, be attributed to organs within the visceral ball where the majority of metabolism occurs in geoducks.

4.4.4 Benzyl Alcohol

Benzyl alcohol was detected in 2 of 18 samples at concentrations of 64 and 100 µg/Kg. The MDL for this compound is 53 µg/Kg. Benzyl alcohol has a wide variety of uses including food preservation, over-the-counter medications, and plastics manufacturing (DOH 1996). Benzyl alcohol was not detected in nearshore sediment samples collected December 2001 within the geoduck study area (King County 2002c).

5.0. SUMMARY AND CONCLUSIONS

Twenty-seven geoducks were collected in April and May 2002 from nine locations within candidate outfall zones for King County's planned Brightwater Regional Wastewater Treatment System. The geoducks were collected as part of a larger survey that was performed to assess geoduck distribution and abundance in the candidate marine outfall zones and the surrounding area.

Nine geoduck tissue samples were submitted for bacterial analysis of three indicator bacteria and 18 samples were submitted for analysis of physical characteristics and tissue chemistry. Chemical analytes included both trace metals and trace organic compounds and chemical analysis was performed on both whole-body and edible-portion tissue samples.

Tissue analysis was performed to establish baseline conditions within the candidate marine outfall zones for chemical body burden and indicator bacteria tissue concentrations. These geoduck tissue baselines may then be used as benchmarks against which results from any possible future monitoring during outfall operation might be compared.

Analytical results from physical, bacterial, and chemical testing indicate the following characteristics of geoducks and geoduck tissue.

- Geoducks in this study ranged in age from 9 to 95 years old with a mean age of 50 and a median age of 54.
- There was no apparent correlation between geoduck age and weight. The mean and median organism weights in this study were 1.15 and 1.16 Kg, respectively.
- Indicator bacteria, including fecal coliforms, enterococci, and *E. coli*, were detected at counts similar to those found in butter clams collected from relatively unimpacted areas in King County. Geoduck bacteria counts were below the FDA safety level for fecal coliforms and *E. coli* in shellfish.
- Twelve of 14 trace metals analyzed were detected in geoduck tissue. With the exception of mercury, there was no apparent correlation between geoduck age and trace metal concentrations. Regression analysis indicates that mercury concentration may increase with organism age.
- Five of six metals for which the FDA provides regulatory or guidance criteria were well-below their respective criteria. Lead concentrations detected in eight of nine whole-body samples exceeded the FDA criterion, by as much as six times. Lead appears to be concentrated in the visceral ball. Lead was not found at elevated concentrations in sediment samples collected from the geoduck study area.
- Six organic compounds were detected in geoduck tissue samples. One of these compounds, benzoic acid is naturally-occurring in shellfish and appears to be present in the organisms as byproducts of metabolic processes. A second compound, bis(2-ethylhexyl) phthalate may have been imparted to samples through handling during tissue

sample processing. Bis(2-ethylhexyl) phthalate was not found at elevated concentrations in sediment samples collected from the geoduck study area.

- Alpha, beta, and gamma (Lindane) isomers of the pesticide BHC (benzenehexachloride), were detected in geoduck tissue samples. There was no apparent correlation between these pesticide concentrations and organism age, lipid concentration or the depth from which the organism was collected. These pesticides have not been detected in sediment samples collected from the geoduck study area. Regulatory or guidance criteria were not found for these pesticides in shellfish.

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