SECTION 5
Summary of Tissue Monitoring Data

This section summarizes bivalve (clam) and macroalgae data collected in 2004 from the ambient and outfall monitoring programs. A summary of data trends observed over the last several years is also included in this section.

Bivalves were collected from seven sites in 2004; three ambient and four outfall sites. Macroalgae samples were collected from eight sites in 2004; three ambient and five outfall sites. A change was made to the biota tissue monitoring program in 2004—fecal indicator bacteria monitoring in shellfish tissues was discontinued. This change occurred as both the State water quality standards and the National Shellfish Sanitation Program guidelines are based upon the amount of fecal indicator bacteria in the water. An evaluation of over 10 years of sampling data indicated that bacteria counts in shellfish were highly variable and could be affected by a variety of factors, such as tidal stage, time of year, and processing. Therefore, bacteria analysis in bivalves was discontinued. All bivalve and macroalgae data are presented in Appendices C and D. Station locator maps are provided in Section 2, along with specific station information such as matrix, parameters, and frequency measured.

A summary of results is provided in this section. Each matrix is discussed separately.

5.1 Bivalve Data Results

Bivalve tissue composite samples consisting entirely of butter clams, *Saxidomus giganteus*, were collected from beaches as a part of the ambient and outfall monitoring programs. Butter clams were collected in August 2004 and analyzed for lipid content, metals, semivolatile organics, pesticides, and PCBs. Shell size was also measured for each clam collected. Clams were collected at beaches near four outfall sites (KSHZ03-Carkeek Park, KSSN05-West Point, LSKS01-Alki Point, MSJL01-Vashon Island) and three ambient stations (JSVW04-Point Wells, KSLU03-Golden Gardens, MTLD03-Normandy Park).

Results are provided in Appendix C and are presented on a wet weight basis unless otherwise noted. Shell lengths of individual clams included in each composite sample are also provided in Appendix C.

5.1.1 Lipids

Bivalve tissue samples were analyzed for lipid content as certain organic compounds have a higher affinity to lipids. Butter clam lipid content ranged from 0.55 to 0.83 percent in 2004, excluding two results. Lipid content in clams from stations KSHZ03 and KSLU03 were 3.49%

and 4.75%, respectively. It is likely that another clam species was inadvertently included in the two composite samples as butter clam lipids are normally less than 1%. Horse clams, which are found at both sites, have much higher lipids (generally greater than 3%) and were likely included in the composite samples. Results from these two sites in 2004 have been excluded from further discussion.

The percent lipid content found in bivalves collected from 2004 was similar to the distribution of lipid content from previous year's data. Figure 5-1 shows a frequency plot of lipid content for butter clams analyzed between 1998 and 2004. These results are similar to the lipid content for butter clams collected in June 1999 from Samish Island (northern Puget Sound) which was 0.4 % (Johnson, 2000).

Clams were collected between mid-July to mid-August from 1998 to 2004 and lipid content was most often between 0.4-0.6% during this period. Reproductive state may affect lipid content with higher lipids occurring prior to spawning and lower lipids after spawning. Butter clam spawning season is in the fall for Washington and it appears that the clams were collected during a stage prior to spawning for most years.

There was no relationship between lipid content, shell size, and age class. Clams collected were at least 50 mm (four years in age) in length when possible in an effort to minimize any age class affect on data analysis.

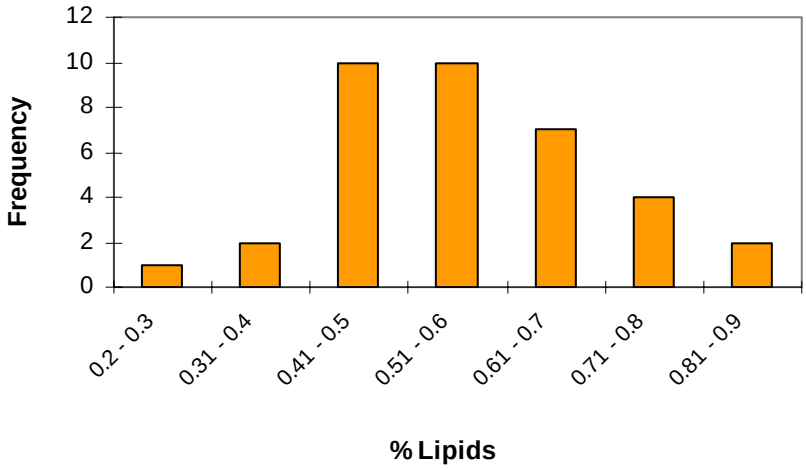

Figure 5-1. Percent Lipids in Butter Clams Collected Between 1998 and 2004

5.1.2 Shell Lengths

Shell lengths were measured for individual clams comprising each composite sample. Individual clam shell lengths ranged from 40 to 90 mm in 2004, with an average shell length of 72 mm. Composite sample average shell lengths ranged from 60 to 82 mm. The table below provides a rough estimate of shell length and the estimated corresponding age class for butter clams as

described in Goong (1999). These estimates of clam age can vary; older clams tend to have more growth variation than younger ones.

Shell Length (mm)	Age Class (yr)
10	0+
20	1+
30	2+
40	3+
50	4+
58	5+
65	6+
70	7+
75	8+
80	9+

Figure 5-2 shows a frequency distribution for composite sample shell lengths collected between 1998 and 2004 and the estimated corresponding age class. The majority of clams analyzed for chemical constituents were nine years or older.

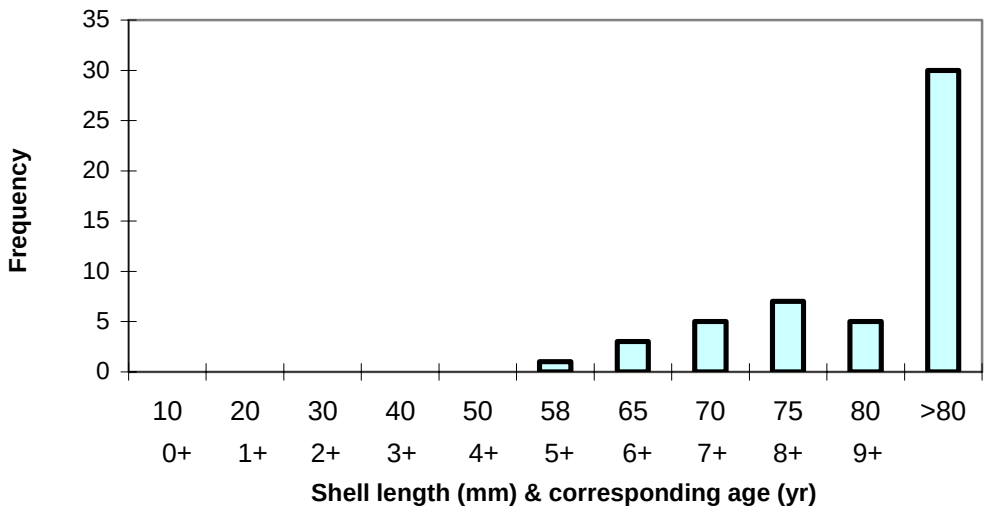

Figure 5-2. Frequency distribution of butter clam shell lengths and corresponding age class for organisms collected from 1998 – 2004.

5.1.3 Metals

Clam tissues from seven sites were analyzed for metals in 2004. In order to discuss long-term temporal trends, the data collected in 2004 will be discussed along with previous years data. Eleven metals were analyzed in clam tissues between 1998 and 2004: arsenic, beryllium,

cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, and zinc. Table 5-1 provides a summary of detected metals in clam tissues. Beryllium was never detected above the method detection limit of 0.02 mg/Kg wet weight. The results for the other 10 metals are presented on a dry-weight basis (the solids content ranged from 14.1 to 21.8%).

In order to evaluate whether there were statistical differences in metal concentrations between sites, data were assessed using a one-way ANOVA at a 90% confidence level. A Tukey post-hoc test was used to rank stations based on differences in clam tissue metal concentrations.

Seven of the 10 metals showed some statistically significant differences between sites, with mercury, selenium, and zinc exhibiting no difference between all sites. Arsenic concentrations were significantly higher at MTLD03 (Normandy Park) than at the other sites, while concentrations were lowest at KSSN05 (West Point) and KSHZ03 (Carkeek Park). Cadmium showed a similar trend to arsenic, with concentrations highest at MTLD03 (Normandy Park). Copper and Lead concentrations were highest at JSVW04 (Richmond Beach). Silver concentrations were significantly lowest at KSSN05 (West Point).

Table 5-1. Descriptive statistics of metal concentrations (mg/Kg dry weight) found in butter clam tissues from 1998-2004.

	Mean ± S.D.	Minimum	Maximum	Median	# of Samples
Arsenic	17.2 ± 3.18	11.3	23.8	17.4	40
Cadmium	0.344 ± 0.062	0.237	0.542	0.324	40
Chromium	2.13 ± 0.883	0.990	4.66	1.91	40
Copper	10.6 ± 3.40	6.81	23.3	9.50	40
Lead	0.687 ± 0.256	0.332	1.50	0.623	40
Mercury	0.039 ± 0.022	<0.004	0.086	0.040	40
Nickel	5.01 ± 0.823	3.32	7.40	4.95	40
Selenium	2.04 ± 0.511	<0.3	3.36	2.02	40
Silver	4.25 ± 2.21	0.550	8.33	4.04	39
Zinc	78.5 ± 9.30	58.0	101	77.7	40

King County metals data were compared to a similar Ecology study which used butter clams collected around Samish Island (Johnson, 2000). The concentrations of arsenic, cadmium, lead, mercury, and selenium from the Ecology study were within the same range as the results obtained from butter clams within King County.

While there are no Washington State criteria for metal concentrations in shellfish, the U.S. Food and Drug Administration (FDA) has established wet weight-based guidance values. The FDA has posted a ‘Level of Concern’ for five metals: arsenic, cadmium, chromium, lead, and nickel. The FDA also posts an ‘Action Level’ for mercury concentrations (FDA, 1995). Food products exceeding the Action Level can not be commercially traded; an important distinction from Levels of Concern. These guidance values differ for children and adults. For comparative purposes, the lower of the two values was chosen. Table 5-2 shows the Level of Concern for the five metals listed above (FDA 1993a, b, c, d, e).

Table 5-2. FDA Levels of Concern in Shellfish Tissues

	mg/Kg wet weight
	Level of Concern
Arsenic	55
Cadmium	3
Chromium	11
Lead	0.8
Nickel	80
	Action Level
Mercury	1.0

All wet weight metal concentrations in bivalves collected by King County were below the levels recommended by the FDA. When concentrations are converted to a dry-weight basis to account for moisture content (as in Table 5-1), some values are over the FDA wet weight guidelines. However, as stated above, all wet weight values were below the FDA guidelines.

5.1.4 Organics

Of the 98 organic compounds analyzed in bivalve tissue between 1998 and 2004, only four have ever been detected: benzoic acid, benzyl alcohol, beta-BHC, and bis(2-ethylhexyl)phthalate. Benzoic acid occurred in all samples at concentrations ranging from 3,218 to 80,368 µg/Kg-dry weight. Benzoic acid is used in the formation of other chemical compounds, as a food preservative, particularly in acidic foods and beverages, a preservative in some toothpastes and cosmetics, and as an anti-fungal agent. It also occurs naturally as it is produced by many plants, with high concentrations found in certain types of berries. Benzoic acid occurs in many animals as it is a degradation product produced by metabolic processes and has always been found in bivalve tissue samples.

Beta-BHC was detected in two samples in 2002: KSSN04 (6.49 µg/Kg-dry weight) and KSSN05 (6.76 µg/Kg-dry weight). Beta-BHC had not been previously detected in bivalve samples at these sites and 2002 was the only year in which this compound has been detected in clam tissues. Beta-BHC was formerly used in the U.S. as an insecticide and is toxic to aquatic organisms when it bioaccumulates.

Benzyl alcohol was detected in only one bivalve tissue sample between 1998 and 2004. A value of 597 µg/Kg-dry weight was detected in the sample from station JSVW04.

Bis(2-ethylhexyl)phthalate was detected in all the samples in 2001, but was most likely the result of laboratory contamination.

5.2 Macroalgae Data Results

Ulva spp., or sea lettuce, grows ubiquitously in Puget Sound during the summer months where there is appropriate substrate. It can be found attached to rocks, pilings, and other algae with typical blade lengths of six inches. Sea lettuce is used by King County as a biomonitor to monitor metal concentrations in water over time. Healthy, attached *Ulva spp.* samples were collected from five outfall (KSHZ03, KSSN05, LSKS01, MSJL01, KSSN04) and three ambient (KSLU03, JSVW04, MTLD03) beach stations in August of 2004. The samples were analyzed for 14 metals commonly monitored for water and sediment quality.

Aluminum, arsenic, cadmium, copper, iron, lead, manganese, nickel, and zinc were detected in all samples collected in 2004. Chromium was detected in all samples except JSVW04. Silver was detected in three samples (JSVW04, KSHZ03, KSLU03) at levels only slightly above the detection limit. Selenium, beryllium, and mercury were not detected in any of the samples (see Appendix D for all 2004 metal data).

Further analysis of metal concentrations in macroalgae was performed to evaluate whether long-term patterns may exist. The King County dataset used in this long-term analysis was limited to the eight stations where metals data were collected in 2004. Macroalgal metals data were compiled from 1996-2004.

The mean concentrations of the 14 metals shown in Table 5-3 have high variability with the exception of arsenic. The levels of arsenic detected in macroalgae samples increased from 2000-2003 and decreased in 2004 (Figure 5-3). These trends appear to occur across all stations and may be indicative of fluctuating arsenic levels in the water column of Puget Sound. The high degree of variability associated with the remaining metals data make it difficult to discern statistically significant long-term temporal trends.

Table 5-3. Summary of 1996-2004 metal concentrations in the macroalga *Ulva spp*.

	Mean metal concentration (mg/Kg DW) ± Standard Deviation
Aluminum	736 ± 653
Arsenic	6.21 ± 1.71
Cadmium	0.552 ± 0.285
Chromium	3.31 ± 3.59
Copper	14.9 ± 26.2
Iron	1241 ± 947
Manganese	65.5 ± 74.8
Nickel	5.79 ± 6.54
Lead	0.921 ± 0.506
Zinc	18.6 ± 26.0

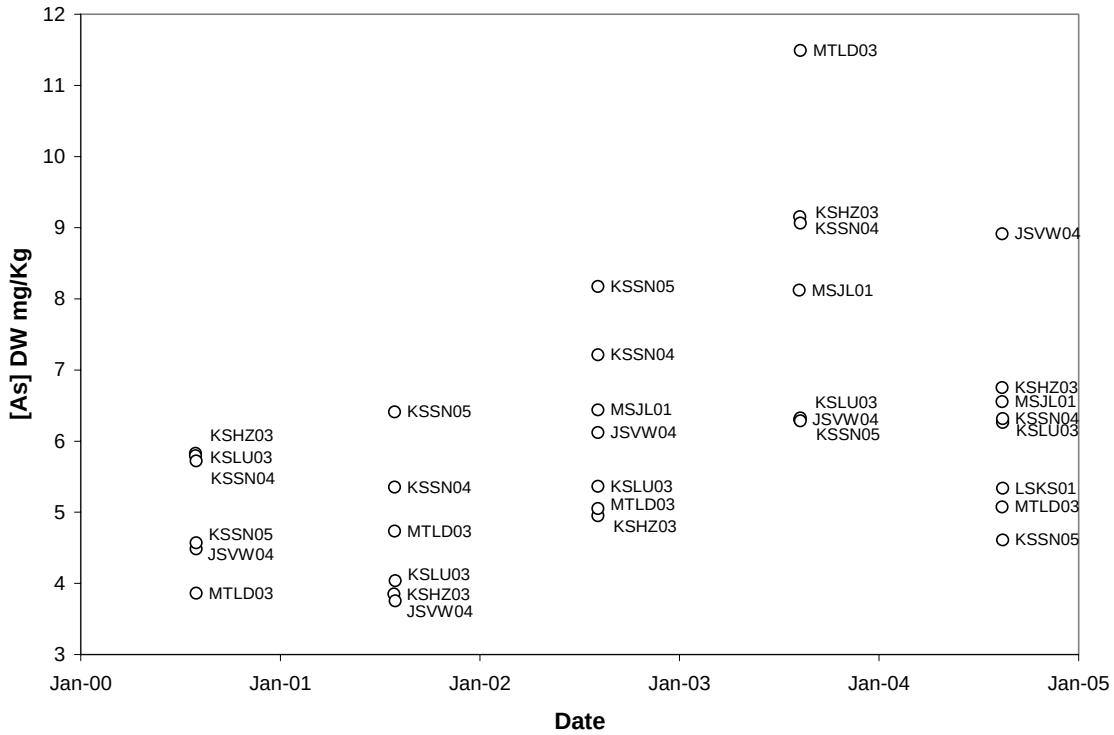

Figure 5-3. Temporal trend of Arsenic in macroalgal samples collected from 2000-2004.

The relatively high levels of aluminum, chromium, iron, and lead in the macroalgae samples suggest fine sediment particles were contaminating the macroalgal samples as these four metals are similarly abundant in Puget Sound sediments (Villares *et al.*, 2005). Another possible indication of the presence of contamination by particulate material is the existence of similar correlations between iron and chromium in the sediment and the algae (Villares *et al.*, 2005). Bryan *et al.* (1985) suggest that related iron/chromium ratios between beach sediments and macroalgae are an indication of sediment contamination as their absorption in seaweeds and sediments are not similar. Figure 5-4 shows the relationship between iron and chromium concentrations from both macroalgae and beach sediments from select King County stations. The ratio of iron to chromium is similar for both macroalgae and sediment as seen in Figure 5-4. These data suggest that fine sediment particles may have adhered to the tissues samples and biased the data, therefore, results may not be indicative of metal concentrations in macroalgal tissues.

Arsenic was not detected in sediments, therefore, the ratio of arsenic to other metals cannot be determined for sediments. Arsenic in sediments in analyzed by ICP instrumentation with a detection limit of 2.5 mg/Kg. In macroalgae tissues, arsenic is analyzed by ICP-MS instrumentation with a detection limit of 0.02 mg/Kg and was detected in the macroalgae samples. Since arsenic was not detected in the sediments, it is not possible to determine whether

the observed arsenic trends shown in Figure 5-4 were found in macroalgae or in sediments but it is likely that effects from particulates would be less for arsenic than other metals.

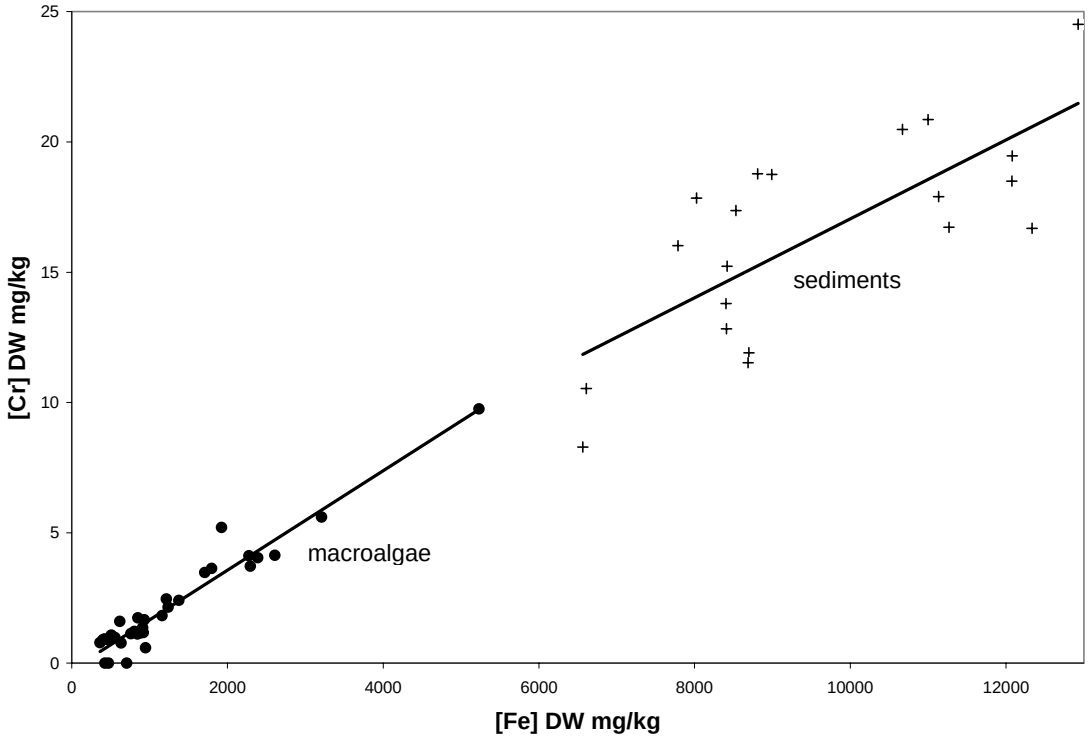

Figure 5-4. The Relationship Between Iron (Fe) and Chromium (Cr) Concentrations Found in King County Macroalgae and Beach Sediments.