Final Project Report-

Characterization of the Cadmium Health Risk, Concentrations and Ways to Minimize Cadmium Residues in Shellfish: Sampling and Analysis of Cadmium in U.S. West Coast Bivalve Shellfish

USDA Cooperative State Research Education and Extension Service
Award No. 2004-51110-02156

Prepared for

Oregon State University
Seafood Research Laboratory
2001 Marine Drive
Astoria, OR 97103

Prepared by

Pacific Shellfish Institute
Olympia, WA 98501

Integral Consulting, Inc.
Mercer Island, WA 98040

Northern Economics Inc.
Anchorage, AK 99501

Hong Kong University of Science and Technology
Clear Water Bay, Kowloon, Hong Kong

And

Pyron Environmental, Inc.
Olympia, WA 98502

August 2008

1.0 TABLE OF CONTENTS

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

ATSDR	U.S. Agency for Toxic Substances and Disease Registry
EC	European Communities
EPA	U.S. Environmental Protection Agency
EPC	exposure point concentration
FDA	U.S. Food and Drug Administration
FAO	Food and Agriculture Organization of the United Nations
HKUST	Hong Kong University of Science and Technology
HC	Health Canada
Integral	Integral Consulting Inc.
ML	maximum limit
NEC	Northern Economic Consultants
NOAEL	no observed adverse effect level
OSUSRL	Oregon State University Seafood Research Laboratory
PDF	probability density function
PRA	probabilistic risk assessment
PSI	Pacific Shellfish Institute
RBA	relative bioavailability adjustment
UCL	95^{th} percentile upper confidence limit of the mean
USDA	U.S. Department of Agriculture
WDOE	Washington State Department Ecology
WDOH	Washington State Department Health
WDFW	Washington State Department of Fish and Wildlife
WHO	World Health Organization

2.0 INTRODUCTION

2.1 PROBLEM IDENTIFICATION

In 2003, Codex Alimentarius, an international commission responsible for setting food standards and guidelines, proposed a 1-part-per-million (ppm or µg/g) wet weight maximum limit (ML) for cadmium in molluscan shellfish The Commission subsequently adopted a ML of 2mg/kg for cadmium in marine bivalve mollusks excluding oysters and scallops, with member states retaining the option to set specific MLs (Codex, 2006). Cadmium is a human health concern because of its ability to accumulate in the liver and kidneys, causing damage at high concentrations. Small amounts of this metal are naturally found in soil, air, and water; however, activities such as mining, smelting, and fuel combustion can increase exposure to humans through food chain biomagnifications processes.

Limited sampling of West Coast commercial shellfish farms indicated that cadmium levels may significantly exceed the 2-µg/g action level and approach or exceed the U.S. Food and Drug Administration (FDA) level of concern of 4.0 µg/g in certain shellfish species and growing areas (USFDA 2005). From 1999 to 2005, several shipments of live oysters from the Pacific Norwest region were barred from entering the Hong Kong market because cadmium concentrations in oyster tissue exceeded the 2 µg/g ML adopted by that government (HKFEHD 2007). Lacking appropriate data, it was not been possible to perform a comprehensive risk scoping and analysis regarding cadmium human health vectors and to thus estimate safe levels of consumption for West Coast-produced shellfish.

U.S. Pacific coast shellfish farmers produce over 100 million pounds of molluscan shellfish annually. The establishment of a 1 or 2 µg/g ML would have significant impacts for molluscan shellfish farmers both regionally and globally. To put this problem in a historical perspective, in 1999 and 2000, multiple shipments of Pacific oysters from British Columbia were rejected by Hong Kong for exceeding the 2 µg/g ML import standard (HKFEHD 2007). More recently, in 2003, a shipment from Hood Canal, Washington (Schallie 2001) was rejected for the same reason. A review of Cd levels in Pacific oysters around Vancouver Island in 2000 by Canada's Department of Fisheries and Oceans found more than 60% of the 81 samples taken had levels of cadmium over 2 µg/g (Schallie 2001). Similarly, following the 2003 Hood Canal shipment rejection, Washington Department of Health processed 25 samples and found cadmium levels ranging from 1.2 to 4.9 µg/g with a median of 2.6 µg/g (WDOH 2003a). In 2005, the Hong Kong Food and Environmental Hygiene Department suspended importation of a shipment of 910 dozen oysters from Hood Canal harvesting area #8 after measuring cadmium concentrations in excess of 2 µg/g (HKFEHD 2007).

This report evaluates the distribution of cadmium in Pacific oysters (*Crassostrea gigas*) and other bivalve shellfish harvested from commercial, recreational and tribal shellfish growing areas located throughout the U.S. West Coast estuaries. The study attempts to identify factors including water quality, sediment chemistry, oyster tissue weight and seasonality that may influence cadmium concentrations and uptake rates in Pacific oysters. The current and potential economic impacts to shellfish growers for cadmium in shellfish as well as the potential human health risk to the general public and high-risk consumer groups are discussed. Results of this research may also be used by the U.S. FDA and the Interstate Shellfish Sanitation Conference (ISSC) when evaluating cadmium MLs proposed by Codex or other countries.

This research project was managed by the Oregon State University Seafood Research Laboratory (OSUSRL) in collaboration with the Pacific Shellfish Institute (PSI), Integral Consulting (Integral), a team of university and private research and extension organizations. OSUSRL is a world-class seafood development and food safety research

institution with a long and successful history in addressing critical research problems affecting the seafood and shellfish industry.

2.2 OCCURRENCE OF CADMIUM IN THE ENVIRONMENT

Cadmium, in pure form, is a soft, silver-white metal found in the earth's crust and detected in small amounts in the soil, air and water. In the environment, cadmium typically occurs as a mineral combined with other elements. Complexes with oxides, sulfides, and carbonates in zinc, lead, and copper ores are most common, while complexes with chlorides and sulfates occur to a lesser extent (ATSDR 1999; NTP 2005).

Cadmium releases into the environment occur as a result of both natural and human activities. Weathering of cadmium minerals contained in rocks is a significant source of these releases to water in rivers and the ocean. Natural releases to air are contributed by forest fires and volcanoes. Mining activities, burning of fossil fuels and of household wastes, application of fertilizers to crops, and industrial sources may also contribute to cadmium levels in the environment.

The primary means of cadmium exposure to the general population is through consumption of food and smoking of tobacco products. According to the United States Food and Drug Administration (USFDA) Total Diet Study, the average lifetime exposure to cadmium from all food sources, excluding shellfish, is 10 µg/person-day (USFDA 1993). Gastrointestinal absorption of cadmium is approximately 5% in most foodstuffs, but may be significantly higher (20% to 30%) in individuals with low body stores of iron, calcium and zinc (Satarug et al. 2004). Cadmium exposure for individuals smoking tobacco products contributes an additional 10 µg/person-day. Approximately 20% to 50% of the cadmium in inhaled smoke is absorbed (USFDA 1993).

Shellfish are known to accumulate significantly higher levels of cadmium due to metallothionein proteins within these organisms that strongly bind to the metal. Results from the 1985 - 1986 USFDA shellfish survey and 1978 National Marine Fisheries Service (NMFS) fish survey reported cadmium concentrations in Pacific oysters ranging from 0.83 to 1.4 µg/g and 1.0 to 2.0 µg/g respectively (USFDA 1993). The consumption of one 50-gram oyster with a cadmium concentration of 2 µg/g would therefore be the equivalent of consuming 100 µg of cadmium.

2.3 HUMAN HEALTH CONCERNS OF CADMIUM EXPOSURE

Cadmium is a human health concern due to its long biological half life (10 to 30 years) and ability to accumulate in soft tissues, especially the liver and kidneys. Long-term chronic exposure to low levels of cadmium are implicated in conditions such as kidney dysfunction, liver disease, lung cancer and skeletal decalcification (Sullivan et al. 1984; Satarug et al. 2000, 2004; Elinder and Jarup 1996; Kuhnlein and Chan 2000; Vahter et al. 2002; USFDA 1993). Evidence suggests that cadmium may also act as a DNA mutagen and possible endocrine disrupter (Jin et al. 2003; Johnson et al. 2003; ATSDR 1999).

Concern over elevated cadmium levels in the environment and human food supply led to the establishment of a provisional tolerable weekly intake (PTWI). The Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee of Food Additives (JECFA) defines the PTWI as the level of intake of an accumulative contaminant which can be ingested without appreciable health risk over a lifetime intake period (WHO 1989). A PTWI of 7 µg Cd/kg of body weight from all sources, or about 60 µg Cd/person-day for a 60 kg person, was established. The current USFDA 3.7 µg/g maximum permitted level (ML) of cadmium contamination, or "Level of Concern," is based on the maximum tolerable daily intake for cadmium (55 µg/person-day) divided by the average intake of molluscan bivalves (15 grams/person-day) (USFDA 1993).

Exceedences of the ML for cadmium in molluscan shellfish tissue also generates human health concerns, especially among populations that consume higher than average quantities of shellfish. Native Americans rely heavily on shellfish for commercial, subsistence and ceremonial purposes. While the U.S. Environmental Protection Agency recommends a fish consumption rate (FCR) of 6.5 g/day for fish and shellfish from estuarine and fresh water for an "average American" (USEPA 1980), two fish consumption surveys conducted by Washington State tribes documented FCRs of 22.3 and 132.7 g/day for shellfish alone (Toy et al. 1996; Suquamish Tribe 2000). With consumption rates up to 20 times higher for certain populations, the potential human health risk of consuming cadmium enriched shellfish may be significant.

2.4 MAGNITUDE OF CADMIUM ENRICHMENT IN SHELLFISH

Over the past 20 years, testing of cadmium concentrations in molluscan shellfish has been limited and patchy throughout Washington State. NOAA's universally recognized "mussel watch" program published cadmium levels for several shellfish species located throughout the United States with 12 sampling stations located in Puget Sound. The mean cadmium concentration in Atlantic oysters (*C. virginica*) from 1986 to 1998 was 0.635 µg/g ranging from 0 to 6.75 µg/g (wet tissue weight) (NOAA 1998).

The Puget Sound Ambient Monitoring Program tested native littleneck clams (*Protothaca staminea*) for trace metal concentrations at 29 recreational harvest sites throughout Puget Sound from 1992 to 1993. Mean cadmium concentrations were 0.19 µg/g (SD = 0.04, n = 20) in 1992 and 0.25 µg/g (SD = 0.08, n = 19) in 1993 with the highest concentration reported at 0.37 µg/g (wet tissue weight) (Patrick 1996).

In 1999, Washington State Department of Ecology tested clams, crab, oysters and mussels for trace metals in the Padilla Bay vicinity in response to Swinomish Tribal Community's concerns over toxics in shellfish. Oysters were strong accumulators of cadmium with concentrations 2 to 7 times higher than clams. Pooled cadmium levels for oysters and clams ranged from 0.2 to 1.4 µg/g (wet tissue weight) (WDOE 2000).

Taylor Shellfish Farms (Shelton, WA) performed independent cadmium testing of various types of molluscan shellfish (clams, oysters, geoduck, mussels) from multiple south Puget Sound locations immediately following Hong Kong's rejection of a shipment of Hood Canal oysters. Mean cadmium concentrations ranged from 0.473 µg/g in farmed geoduck to 0.832 µg/g in oysters. Of the five different oyster species tested, mean cadmium levels ranged from 0.70 µg/g in Olympia oysters to 0.97 µg/g in Pacific oysters (WDOH 2003b).

The Alaska Department of Environmental Conservation (ADEC) tested cadmium concentrations in molluscan shellfish from 1996-2001. Mean Cd concentrations for various species were as follows: butter clams (0.12 µg/g, n=71), razor clams (0.16 µg/g, n=36), geoduck clams (0.18 µg/g, n=71), mussels (0.92 µg/g, n=67) and oysters (2.9 µg/g, n=368). Mean Cd concentrations for Pacific oysters from Southeast Alaska, Cook Inlet and Prince William Sound were 2.97 µg/g, 1.97 µg/g and 3.40 µg/g respectively (Dewey, personal correspondence).

The exact magnitude of cadmium enrichment in Pacific west coast shellfish was unknown until completion of the current study. The scattered and limited sampling of commercially farmed molluscan shellfish described above indicated that cadmium levels significantly exceeded Codex's proposed 1 µg/g international action level and in certain cases approached or exceeded the US FDA's 3.7 µg/g Level of Concern. Based on these data, it appeared that a large portion of Washington and Alaskan Pacific oysters already exceeded Codex's proposed 1 µg/g ML for cadmium in molluscan shellfish, which could result in considerable export restrictions for this commercially important product.

3.0 OBJECTIVES

The research objectives of this study were broadly focused on the identification of the spatial and temporal extent of cadmium enrichment in bivalve shellfish, with emphasis on Pacific oysters, to examine the physiological mechanisms of enrichment, assess management measures, and to determine economic impacts. The specific objectives were:

- To complete a comprehensive sampling effort of cadmium concentrations in Pacific oysters throughout U.S. west coast growing areas.
- To perform laboratory experiments on the uptake and depuration of cadmium by oysters and to develop a model to predict the biochemical behavior of cadmium in oyster tissue under different environmental conditions.
- To define geographic locations, culture methods, shellfish species, harvest times and additional factors that affect cadmium concentrations in an effort to minimize cadmium residues in shellfish products.
- To conduct a preliminary human health risk assessment on consumption of west coast oysters based on data collected in this study and validated probabilistic models and approaches from the literature.
- To identify the economic risk to the oyster industry given the currently accepted maximum permitted level (ML) of cadmium in molluscan shellfish.
- To provide outreach and extension services to the shellfish industry, high risk consumer groups, regulatory agencies, and the scientific community.

4.0 METHODS

The team members designed a field sampling plan and quality assurance project plan (FSP/QAPP; PSI 2004) to complete a comprehensive sampling effort of cadmium concentrations in Pacific oysters (*Crassostrea gigas*) throughout growing areas of the U.S. Pacific Coast from California to Alaska. The FSP/QAPP defined geographic locations, culture methods, harvest times, and additional factors that affect cadmium concentrations in an effort to minimize cadmium residues in oyster products.

4.1 SAMPLING APPROACH

The following sections describe the sampling approach designed to assess the general distribution of cadmium concentrations in tissue of Pacific oysters and the subsequent selection of specific sampling stations for determination of seasonal trends. At each sampling station, samples for oyster tissue composites, sediment sample composites, and surface water grab samples were collected for the analyses of cadmium, zinc, and conventionals (i.e. total suspended solids [TSS] for water samples; total organic carbon [TOC], total solids and grain size for sediment samples).

4.1.1 Geographic Distribution of Tissue Cd Concentrations

To assess the general distribution of cadmium concentrations in tissue of Pacific oysters, in 2004 a total of 41 sampling stations were selected, including four shellfish-growing areas in California, three areas in Oregon, three areas in Alaska, 28 areas located throughout Puget Sound, and three areas in Washington coastal estuaries (Figure 1, Christy 2005). Sites were selected based on the presence of bottom-cultured oysters, geographic

representation, and the importance and/or size of the commercial, tribal or recreational shellfish growing area. Site locations were assigned a unique station identification code, and their geographical coordinates were recorded using a global positioning system (GPS).

4.1.2 Evaluation of Contributing Sources & Factors

Based on the results of the 2004 region-wide sampling event, seasonal collection of oyster tissue, seawater, and sediments was conducted from Winter 2005 to Fall 2005 (Winter 2006 in California) at 10 locations to determine harvest times (seasonality) and additional factors that may impact cadmium concentrations. Each of the five Washington locations were selected to represent a distinct geographic region [south Puget Sound (SPS), Hood Canal (HC), north Hood Canal (NHC), north Puget Sound (NPS), coastal estuaries (CE)]. In addition, two stations were selected in California (Humboldt Bay and Tomales Bay), two stations in Oregon (Tillamook Bay and Netarts Bay) and one station in Alaska (Windy Bay) (**Error! Reference source not found.**).

Oyster tissue, seawater and sediments were collected in January, April, July and October to test for tissue metal concentrations (Cd and Zn), total and dissolved metals in seawater (Cd and Zn), metals in seston (Cd and Zn), total suspended solids, plankton, sediment metal concentrations (Cd and Zn), total organic carbon, and sediment grain size. In addition, to better understand the effect of growth rate, tissue weight and corresponding cadmium concentration, cages of oyster seed of similar age, size and family were distributed to five Washington locations from April, 2005 to October, 2005.

Additional cages of oyster seed were placed at six locations (five in Washington and one in Oregon) in April, 2005 and shipped to Dr. Wen Wang at The Hong Kong University of Science & Technology in July and October, 2005. Laboratory experiments were performed to test oysters for cadmium body burdens, subcellular cadmium concentrations, Metallothionein (MT) concentrations, cadmium clearance rates, assimilation efficiencies (for different food types and diatom concentrations), dissolved uptake rates (rate at which oysters take up dissolved metals in seawater) and efflux rates. Experiments were conducted shortly after sample receipt in July and October, 2005.

Further experiments were conducted to evaluate ways to minimize tissue cadmium concentrations in Pacific oysters. To test the impact of culture method and oyster ploidy on Cd concentration, diploid and triploid seed were placed at four Washington locations in adjacent on-bottom bags or off-bottom aqua-purses between April, 2006 and August, 2006. In February, 2006, Oregon State University Seafood Research Laboratory, in cooperation with Goose Point Oyster Company, conducted separate field experiments in Willapa Bay to test the impact of various processing techniques (jarring, cold storage, high pressure processing) on cadmium concentration. To determine how overall species selection impacts Cd concentration, various molluscan shellfish types including clams (geoduck, manila, butter), mussels, and oysters (Olympia, Kumamoto, Virginica, European Flat) were sampled at five Washington locations in April 2006.

In December 2007, Pacific Shellfish Institute conducted additional field sampling at two Washington locations to assess the effect of including or draining oyster nectar (liqueur) in homogenized samples prior to analyzing tissue cadmium concentrations.

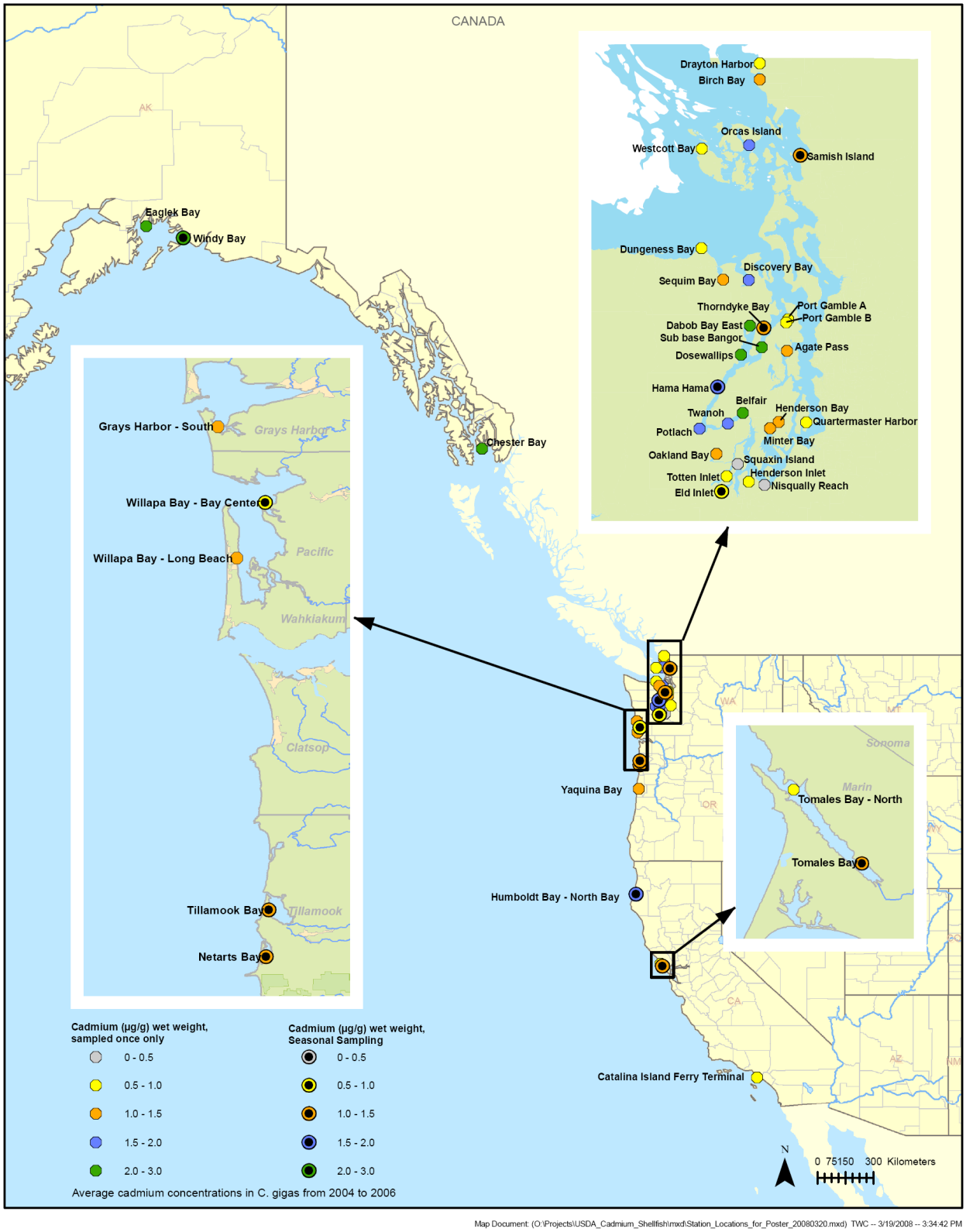


Figure 1. Cadmium sample locations and average tissue concentrations (µg/g wet weight).

4.2 SAMPLING METHODS

4.2.1 Tissue Samples

Pacific oysters were collected during low tide along a 50 meter transect positioned parallel to the waterline at the 0 mean low low water (MLLW) tidal elevation. Ten individual oysters were sampled at each 10 meter increment along the transect line for a total of 60 oysters according to the field sampling standard operating procedures (SOP) (Appendix xxx). All specimens were intact (i.e. tightly closed, unbroken shell) and of similar size (4-6 inches in length).

Oysters were scrubbed with a plastic bristle brush and rinsed on-site using local seawater to remove sand and debris. Biofouling was removed by hand to the best extent possible. Oysters were rinsed a second time in local seawater prior to being systematically divided into three composites of 20 and placed into sealable plastic bags (Figure 2)

Figure 2. Left, field-cleaning and processing of oysters prior to transport to analytical laboratory. Below, typical shellfish farm in Hood Canal, Washington, collection of sediment sample.

Similar protocols were followed for the collection of additional types of shellfish including clams, mussels, and intertidal geoduck. Butter and manila clams were harvested along a transect positioned within their naturally occurring tidal elevation. Mussels were harvested from the upper meter of five-meter lines suspended from a 57 X 9.6 meter mussel raft anchored in place approximately 100 yards from the shoreline. Mussels were collected along approximate 10 meter increments spanning the length of the raft. Geoducks were harvested intertidally by farm personnel using techniques and equipment implemented during normal harvest operations. Composites of three geoducks each were collected and bagged on-site immediately after harvest.

Shellfish samples were maintained at 4ºC and driven within 24 hours or shipped overnight via FedEx to AmTest Laboratories[1]. Chain-of-custody (COC) forms accompanied shellfish samples from the field to the laboratory.

4.2.2 Sediment Samples

Sediment core samples were collected along the same transect line used to harvest oysters (Figure 2). The top 10 cm of sediment were collected at six points along the transect using a 6-inch- diameter section of PVC pipe. Each of the six cores was placed into a stainless-steel bowl and homogenized with a stainless-steel spoon. All sampling equipment was decontaminated prior to use in the field. The homogenized sediment was scooped into pre-cleaned laboratory sample glass jars for analysis, sealed, labeled, and placed in coolers with ice at 4ºC and delivered within 24 hours or shipped overnight to Am Test Laboratories.

4.2.3 Surface Water Samples

Seawater samples were collected one hour prior to the peak of the incoming high tide as close to the transect line as possible. Samples were taken approximately one meter off the bottom of the shellfish bed using 2L HDPE bottles pre-cleaned with Liquinox® soap and deionized water. Seawater was distributed into three pre-cleaned high-density polyethylene (HDPE) bottles containing preservatives to be analyzed for total and dissolved cadmium and zinc, and total suspended solids. All samples were held at 4ºC and delivered within 24 hours or shipped overnight to Am Test Laboratories.

Because many of dissolved cadmium in seawater measurements were undetected at the 0.020µg/L detection limit, surface water samples collected in October 2005, were sent to Brooks Rand LLC [2] for trace metal analysis at the 0.006µg/L detection limit.

An additional 100-ml surface water sample preserved in 1% Lugol's iodine solution was also collected to assess phytoplankton abundance and species composition. Samples were allowed to settle overnight prior to being concentrated 10-fold and viewed under an Olympus® inverted microscope using a 0.1 ml Palmer-Maloney counting chamber.

---

[1] AmTest Laboratories, 14603 NE 87th Street, Redmond, Washington 98052 is accredited for environmental analyses by the Washington State Department of Ecology.

[2] Brooks Rand LLC, 3958 6th Avenue NW, Seattle, Washington 98107 is accredited for environmental analyses by the Washington State Department of Ecology.

4.2.4 Seasonal Analysis of Seawater Temperature and Salinity

To evaluate seasonal changes in seawater temperature, salinity and pH in Washington State, existing data (1990 to 2000) was taken from Washington Department of Ecology's (WDOE) marine water quality monitoring searchable database (WDOE, 2005). Surface (0.5 m - 2.5 m) and depth (9.0 m - 11.0 m) measurements from January, April, July and October were extracted from five WDOE monitoring locations (Eld Inlet-Flapjack Point; Hood Canal-Eldon, Hamma Hamma River; Hood Canal-King Spit, Bangor; Bellingham Bay-Point Frances; Willapa Bay-Toke Point) to represent conditions at Eld Inlet, Hamma Hamma, Thorndyke Bay, Samish Bay and Willapa Bay-Bay Center respectively. Only non-provisional data with a data quality code of 1 (state of art method, adequate QC) were used for analysis.

4.2.5 Oyster Seed Experiments

4.2.5.1 Growth Rate

In April 2005, 1,200 triploid, 6 mm long oyster seed derived from Willapa Bay, Washington wild broodstock were collected from a floating upwelling nursery facility in Oakland Bay, Washington. These seed were a cross between a selected diploid and inbred naturally produced tetraploid families. They were spawned at a hatchery in Hood Canal, Washington mid-late summer of 2004 and sent to a nursery in Hawaii for 4-6 weeks and then returned to the nursery in Oakland Bay. During hatchery growout all seed were fed Isochrysis sp. Collected seed were split equally into 5 groups of 240 individuals and transplanted to five Washington locations (Eld Inlet, Hamma Hamma, Samish Bay, Thorndyke Bay and Willapa Bay) for the purpose of conducting a growth rate and cadmium uptake experiment (Table 4-1). All seed were placed into on-bottom cages that were positioned at the 0-m MLLW line. Prior to being placed in the field, and in July and October 2005, length, depth and total weight measurements (unshucked) were collected on three composites of 30 oysters each. Oyster composites were sent to AmTest Laboratories where total shucked weight measurements were taken and tissues were tested for Cd and Zn. The measured growth rates for each site were also used in the biokinetics model.

4.2.5.2 Uptake and Depuration of Cadmium

Sample materials required to perform laboratory experiments on cadmium uptake and depuration at the Coastal Marine Laboratory at the Hong Kong University of Science and Technology (HKUST) were obtained in April 2005 when a second group of 2,000 triploid, 9.5 mm long seed were collected from the Oakland Bay nursery. These were split into six groups of 333 individuals, placed into mesh seed bags contained within on-bottom cages and transported to five Washington sites (Eld Inlet, Hamma Hamma, Samish Bay, Thorndyke Bay and Willapa Bay) and one Oregon site (Netarts Bay) (Table 4-1). All seed in this group were a cross between a diploid family and a chemically induced tetraploid family. In July 2005, after a 3-month acclimation period, three composites of 30 oysters each were sent from each of the 6 locations to AmTest Laboratories for Cd analysis. The remainder were shipped to HKUST along with an assortment of oysters ranging from 13 to 152 mm in length for laboratory experiments on cadmium uptake and depuration (Appendix A).

A second group of seed oysters was collected in October 2005 and sent to Hong Kong for additional cadmium uptake and depuration experiments. These included diploid seed from Humboldt Bay, California (HUM, and diploid and triploid seed from farm sites in Oakland and Thorndyke Bays. This second group was used to determine if there were any physiological differences between the triploid and diploid populations (Appendix A).

4.2.5.3 Additional Experiments – Culture Technique, Ploidy, Processing & Oyster Liqueur

Figure 3. Experimental plot at Hood Canal location designed to test the impact of culture method and oyster ploidy on oyster Cd concentration.

Culture Technique & Ploidy: To assess the impact of culture method and oyster ploidy on Cd tissue concentrations, an experiment was initiated in April, 2006 (Figure 3). Diploid and triploid oyster seed (3 to 6 mm) in length were placed at four Washington locations [Eld Inlet (SPS), Samish Bay (NPS), Willapa Bay (CE) and central Hood Canal (HC)] using two different culture methods: bag-on-bottom (BOB) (9 mm mesh size) and aqua-purses (AP) (8 mm mesh size). A total of 6 bags and 6 aqua-purses were placed at each location. Of the 6 bags and 6 purses, half contained diploid seed (100 seed/bag or purse) and the other half contained triploid seed (100 seed/bag or purse). Seed (3 composites of 40 oysters/each) were initially sent to AmTest Laboratories to be tested for Cd concentration in late April and again in mid-August (3 composites of 30 oysters/each) for an experimental duration of 117 days. Individual length measurements and unshucked composite weights were also recorded in mid-August.

Processing: OSUSRL, in cooperation with Goose Point Oyster Company, conducted separate field experiments in Willapa Bay to test the impact of processing techniques (jarring, cold storage, high pressure processing) on cadmium concentrations. A cohort of 160 Pacific oysters (*C. gigas*) was harvested on February 2006 from an oyster farm in Willapa Bay, Washington. All oysters were the same age (2.75 yr) and from the same growing bed. The oysters were randomly sorted into 4 groups: shucked (S), shucked and drained (SD), shucked, drained, washed and jar-packed (S-WJ), and high pressure processed (HPP). All glass jars, lids and equipment used to hold shucked oysters were washed with dilute nitric acid in deionized water to remove any bound metals, and then thoroughly rinsed with deionized water prior to use. Oyster shucking knives were washed with dilute nitric acid and deionized water in between each composite sample of 5 oysters.

- *Shucked Oysters (Group S).* Group S consisted of 20 oysters that were shucked into 4 stainless steel bowls, resulting in 5 oysters per bowl. Any fluid that was released from the oyster shell during shucking was also collected in the bowls. Following collection, the composite samples of 5 oysters each were weighed and emptied into labeled Ziploc® Freezer Bags. The bags were sealed and kept in ice-filled coolers for several hours while the rest of the samples were prepared and during transport from the processing facility to the OSUSRL, a distance of about 45 mi. Following arrival at the OSUSRL, each composite sample of 5 oysters was treated as described in the *Cadmium Analysis* subsection.

- *Shucked and Drained Oysters (Group SD).* Group SD consisted of 20 oysters that were shucked in sets of 5 into 4 stainless steel colanders. The colanders allow for excess fluid from inside the oyster shell to drain off, and are commonly utilized at the processing facility. The 4 composites of shucked and drained oysters were collected in labeled Ziploc® Freezer Bags and the weight of each composite was recorded. The bags were then sealed and held in ice-filled coolers while the rest of the samples were processed and during transport to the OSUSRL. All composites were then treated as described in the *Cadmium Analysis* subsection.

- *Shucked, Drained, Washed and Jar-packed Oysters (Group S-WJ).* Group S-WJ consisted of 60 oysters that were shucked and drained into stainless steel colanders in sets of 5, resulting in 12 composite samples. Colanders were washed with a dilute nitric acid solution and rinsed with deionized water between groups of composite samples. Each composite sample was individually weighed (reported as initial weight) and then washed in aerated water for 3 min at 13.3°C, according to the typical washing procedure carried out at the processing facility. Following the washing step, the composite oyster samples were packed into acid-washed, labeled glass jars that were topped off with tap water normally used at the facility for this purpose. After all 12 composites were washed and jar-packed, the sealed jars were held in an ice-filled cooler until the rest of the samples were processed and during transport to the OSUSRL. Following arrival at the OSUSRL, 4 of the 12 glass jars were opened (day 0) and drained for 2 min over a U.S. standard No. 8 sieve (stainless steel) slanted at a 20 degree angle. The sieve was washed with dilute nitric acid and then rinsed with deionized water before draining each jar. The drained oyster samples were then weighed (reported as final weight) and treated as described in the *Cadmium Analysis* subsection below. The remaining 8 jars were held in a walk-in cooler set at 1.5°C. At both day 5 and day 10, 4 jars were removed from the cooler and treated in the same manner as the jars opened at day 0.

- *High Pressure Processed Oysters (Group HPP).* Group HPP consisted of 60 oysters that were each wrapped with a 2 cm plastic shrink-wrap band and then treated with high hydrostatic pressure (300 MPa, 90 sec), which causes a release of the oyster adductor muscle and eliminates the need for shucking (He et al., 2002; Martin and Hall, 2006). Following HPP, the shrink-wrap bands were cut to allow the shell to open and the oyster tissues were collected in sets of 5 in stainless steel colanders. The colanders were washed with a dilute nitric acid solution and rinsed with deionized water between groups of composite samples. The resulting 12 composite samples were then subjected to washing, jar-packing and draining at days 0, 5 and 10, as described above for Group S-WJ, beginning with "Each composite sample was individually weighed..." Each composite was blended for 1 min and then placed into a Ziploc® Freezer Bag. Homogenized composite samples were held at -80°C. Representative portions (~20 g) of each composite oyster sample were placed into separate Ziploc® Freezer Bags. The representative samples were then packaged in coolers with ice packs and shipped by FedEx overnight to AM Test Laboratories in Redmond, WA, for Cd analysis.

Oyster Liqueur: In December, 2007, oysters were collected from two Puget Sound locations (south Puget Sound and central Hood Canal) in order to assess the effect of including or draining oyster nectar (liqueur) in homogenized samples prior to analyzing tissue cadmium concentrations. At each of the 2 locations, 6 composites of 20 oysters each were harvested according to methods described in Section 4.2.1 for Tissue Samples and driven to AmTest Laboratories in Redmond, Washington for Cd analysis.

4.3 FIELD QUALITY ASSURANCE

4.3.1 Field Quality Control Procedures

Due to the large number and geographic distribution of stations, shellfish growers, tribal biologists, and personnel from the Washington Department of Fish and Wildlife and Pacific Shellfish Institute assisted with sample

collection. Sampling materials and detailed SOPs were provided to each participant in order to maintain consistency between locations. All sampling and analytical procedures were performed in accordance with guidelines and protocols published by the USFDA (1993), Puget Sound Water Quality Action Team (PSEP 1997), and AmTest Laboratories' SOPs. The QAPP was reviewed by the Washington State Department of Health Office of Environmental Health Assessment (OEHA) prior to initial sample collection (PSI 2004).

4.3.2 Consistency of Data Collection Methods

Samples were collected using appropriate methods. All participants used the same sampling equipment and followed SOPs provided by PSI, with the exception of samples collected on June and July 2004 at Tomales Bay and Humboldt Bay, California, respectively. Oysters at these locations were shucked at the field lab and were frozen before being sent to AmTest for analyses. However, analytical results for these samples fell well within the lower end of cadmium concentration range for all other samples collected at the same site, indicating that the nonstandard sampling procedures did not contribute to possible sample contamination. A review of field notes for subsequent dates did not reveal issues in sample collection procedures, and procedures were consistent between field sampling events and sampling teams.

4.4 LABORATORY METHODS

4.4.1 Tissue Samples

Tissue samples were processed at AmTest Laboratories within 48 hours of sample receipt. Oysters were individually scrubbed with a soft bristle brush under cool running tap water. After any remaining debris was removed, oysters were rinsed a second time with deionized water. Each composite of 20 oysters was shucked, weighed, and homogenized (tissue and liquor) in a clean blender fitted with stainless-steel blades. Composites were transferred into glass jars and held at -18ºC until further processing. Homogenized tissue samples were then tested for cadmium and zinc using inductively coupled plasma-atomic emission spectrometry (ICP-AES; EPA Method 200.3/6020), or graphite furnace atomic absorption spectrometry (GFAAS; EPA Method 200.9/7131). Tissue cadmium concentrations were reported in µg/g (ppm) based on wet tissue weight.

4.4.2 Sediment Samples

Sediment samples were processed at AmTest Laboratories within 48-hours of sample receipt. Homogenized samples were analyzed for grain size distribution according to the Active Standard Test Method (ASTM) for Particle-Size Analysis of Soils Method D422. Percent Total Organic Carbon was measured according to SW-846 Method 9060 at a detection limit of 0.05%. Total Solids and Total Volatile Solids were measured according to Methods described in the *Recommended Guidelines for Measuring Metals in Puget Sound Marine Water, Sediment and Tissue Samples* (PSEP, 1997) at a detection level of 0.01%. Sediment samples were digested by open-vessel microwave according to U.S. EPA's Solid Waste Manual (SW-846) Method 3050B and analyzed for cadmium and zinc according to Method 6010B using inductively coupled plasma-atomic emission spectrometry (ICP-AES). The ICP-AES instrument detection limit was 0.05µg/g for cadmium and 0.10µg/g for zinc.

4.4.3 Surface Water Samples

Seawater samples were processed at AmTest Laboratories within 48-hours of sample receipt. Seawater samples to be analyzed for total cadmium and zinc were digested according to EPA Method 3010 and analyzed according to EPA Method 200.7 at detection limits of 0.0005 mg/l and 0.001 mg/l respectively. Samples were analyzed for dissolved metals also using EPA Method 200.7 at the same detection limits. Total Suspended Solids were

measured using EPA Method 160.2 at a detection limit of 1 mg/l. Oyster liqueur was analyzed for cadmium in November, 2007, using EPA Method 213.2 at the 0.00002 mg/l detection limit.

Beginning in January, 2005, seawater samples that resulted in undetectable total and dissolved cadmium concentrations at the 0.0005 mg/l limit were reanalyzed using EPA Method 213.2 at the lower detection limit of 0.020μg/L. In addition, seston was tested for cadmium and zinc using EPA Method 2540B at the 0.020μg/L detection limit. Because a majority of the seawater samples analyzed for dissolved cadmium still remained undetected even at the 0.020μg/L detection limit, samples collected in October, 2005, were sent to Brooks Rand Laboratories for trace metal analysis at the 0.006μg/L detection limit.

4.5 DATA QUALITY ASSURANCE

All sampling and analytical procedures were performed in accordance with guidelines and protocols published by the USFDA (1993), Puget Sound Water Quality Action Team (PSEP, 1997), and AmTest Laboratories' SOPs. Quality assurance/quality control standards included the use of digestion blanks, duplicates, spiked test portions, appropriate standard reference material and recovery calculations. One blank, duplicate, and matrix spike was performed for every 10 samples. Less than a 20% difference was allowed between duplicate samples. Greater than 80% recovery was required for both matrix spikes and standard reference materials.

4.5.1 Data Analyses

Data were statistically analyzed using Microsoft® Office Excel. Prior to carrying out any statistical analyses, the significance value was set at $p < 0.05$. The chi-squared test of normality, F-test and Bartlett's test for multiple variances were used to test for normality and homogeneity of variances. For normally distributed data, statistically significant differences between 2 populations were detected using the t-test or paired t-test. For comparisons between multiple populations, the one-way analysis of variance (ANOVA) was applied. If the ANOVA showed a significant difference, Tukey's multiple comparison method was conducted to identify the pairs of means that were significantly different.

Non-normally distributed data were analyzed using nonparametric statistical techniques. The Wilcoxon Signed Rank Sum Test and the Wilcoxon Rank Sum Test were used to identify significant differences between 2 populations for matched pair data and independent data respectively. The Friedman Test and Kruskal-Wallis Test were applied for detecting differences between multiple populations for blocked and independent data respectively.

Correlation analysis was performed to evaluate the nature and strength of relationships. The Pearson coefficient of correlation (r) or Spearman rank correlation coefficient (rs) were reported for normally and extremely non-normally distributed data. Spatial results were geographically depicted using ArcView® software.

4.5.2 Data Validation

The analytical results from AmTest and Brooks Rand were validated by Pyron Environmental (Olympia, WA)(Appendix D). Because the laboratory reports from AmTest did not include the performance-based in-house control limits for the QC analyses, control criteria specified in the U.S. EPA's Contract Laboratory Program (CLP) National Functional Guidelines for Inorganic Data Review (USEPA 2004) were applied to evaluate the data quality. The overall assessment of data usability, based on the information presented in the laboratory reports by AmTest and Brooks Rand, determined that cadmium data were acceptable for use as qualified in Pyron's data validation reports (Pyron 2006a, b).

4.5.3 Cadmium Data Evaluation

4.5.3.1 Data Sources

Data were evaluated to determine whether QA/QC data are available to provide data quality information. As described in Section 4.5.2, data validation of cadmium in oyster tissues were deemed acceptable based on EPA's CLP National Functional Guidelines for Inorganic Data Review (USEPA 2004), since the laboratory reports did not include the performance-based in-house control limits for the QC analyses.

4.5.3.2 Analytical Methods and Detection Limits

Methods were evaluated for appropriateness and sensitivity. It was determined that the detection limit for cadmium of 0.0005 µg/g tissue wet weight is adequate for risk-based evaluation.

4.5.3.3 Data Quality Indicators

Laboratory validation reports were reviewed for data quality issues. All tissue and sediment samples were detected without any qualifiers. Therefore, there was no need to reject or exclude any tissue or sediment data from the database. However, dissolved cadmium in surface water samples was frequently undetected due to high analytical detection limits set at AmTest Laboratories. Because of inadequate sample processing protocols, these specific analytical results were rejected. Surface water samples from the subsequent sampling event were then sent to Brooks Rand for trace metal analyses. Dissolved cadmium was detected in all samples, and no data were rejected.

5.0 FIELD OBSERVATIONS OF CADMIUM IN TISSUE, WATER, SEDIMENT & ADDITIONAL CONTRIBUTING SOURCES AND FACTORS

5.1 GEOGRAPHIC DISTRIBUTION IN SHELLFISH TISSUE

In September, 2004, a total of 117 oyster composites were collected at 39 sites throughout California, Oregon, Washington and Alaska. Two additional sites, Eaget Bay, AK and Catalina Ferry, CA, were sampled in January 2005. Tissue Cd concentrations ranged from 0.44 µg/g in Tomales Bay, CA to 2.49 µg/g in Windy Bay, AK with a mean and standard deviation of 1.24 ± 0.58 µg/g (**Error! Reference source not found.**).

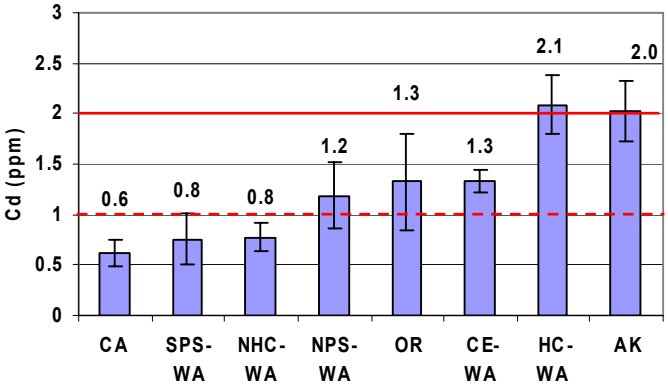


Figure 4. Mean oyster tissue Cd concentration by geographic region from data collected during initial Fall 2004 survey effort. The dotted red line indicates CODEX's proposed 1 µg/g ML and the solid red line indicates Hong Kong's 2 µg/g import restriction.

The Washington data were grouped into five distinct geographic regions according to cadmium distribution: south Puget Sound (SPS), north Puget Sound (NPS), Hood Canal (HC), north Hood Canal (NHC) and coastal estuaries (CE). South Puget Sound (9 sites) extended from Eld Inlet to Quartermaster Harbor, north Puget Sound (9 sites) from Agate Pass to Drayton Harbor and west to Sequim Bay, Hood Canal (7 sites) from

Belfair State Park to Bangor, north Hood Canal (3 sites) from Thorndyke Bay to Port Gamble, and coastal estuaries (3 sites) from Grays Harbor to Willapa Bay.

Mean cadmium concentrations for CA, OR, AK and 5 WA regions, and their relationship to the proposed Codex 1 µg/g ML for cadmium in molluscan shellfish and Hong Kong's 2 µg/g import restriction are displayed in Figure 4. Of the 117 composites tested, 55.6% exceeded 1 µg/g and 17.1 % exceeded 2 µg/g. Of those composites exceeding Hong Kong's 2 µg/g import restriction, 95.0% were located in Alaska and Hood Canal, Washington. Of the 21 Hood Canal composites, 71.4% exceeded the 2 µg/g limit. Of the 6 Alaska composites, 66.7% exceeded the limit.

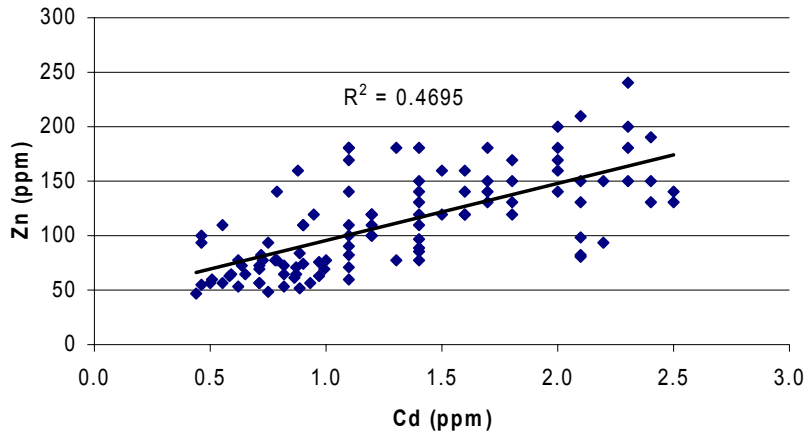


Figure 5. Relationship between Cd and Zn in oyster tissue sampled during the September 2004 sampling effort (n=107). Initial California composites collected in June and July 2004 were not tested for Zn (n=10).

Oyster tissue was also tested for zinc since the two metals are often associated together in the environment and evidence suggests that a diet rich in zinc may offer considerable protection against cadmium accumulation in animal and human subjects (Sullivan et al., 1984). Cadmium concentrations in adult oyster composites collected from the initial sampling effort in September 2004 were positively correlated with zinc concentrations in Pacific oysters (r_s =0.72, p = 0) (Figure 5). Zinc concentrations ranged from 47 µg/g at Squaxin Island, Washington to 240 µg/g at Sub-base Bangor, Washington with a mean and standard deviation of 111.3 ± 44.2 µg/g. Not included in Figure 5 but worth noting, are tissue composites analyzed for Cd and Zn in January 2005 at the Catalina Ferry Terminal. While mean Cd concentrations were low (0.75 µg/g), Zn concentrations averaged 719.3 ± 179.2 µg/g.

5.1.1 California

The Pacific Shellfish Institute coordinated and implemented the sampling plan for the collection of oyster tissue composite samples, sediment composite samples and surface water samples from September 2004 to February 2006 (PSI 2004). Ten of the California oyster tissue composite samples were collected in June and July 2004, prior to the development of the Standard Operating Procedures (SOP) for tissue sample collection established for this project, but were incorporated into the database because of insignificant deviations from the SOP and QAPP.

A total of 10 oyster composites were collected at 3 California sites during the initial sampling effort. Composites were collected in June and July 2004 prior to the development of the SOP. The average tissue Cd concentration was 0.65 ± 0.12 µg/g and ranged from 0.44 µg/g at Tomales Bay to 0.77 µg/g at the Tomales Bay North site.

When including all oyster composites tested for both the initial sampling effort in September 2004 and all additional sampling through Fall 2006 (n=37 from 4 sites), the average tissue Cd concentration was 1.27 ± 0.82 µg/g and ranged from 0.44 µg/g at Tomales Bay (June 2004) to 3.93 µg/g at Humboldt Bay (February 2006).

5.1.2 Oregon

A total of nine oyster composites were collected at 3 Oregon sites during the initial Fall 2004 sampling effort. The average Cd concentration was 1.33 ± 0.48 µg/g and ranged from 0.75 µg/g at Tillamook to 2.04 µg/g at Netarts Bay.

When including all oyster composites tested for both the initial effort and all addition sampling through Fall 2006 (n=39 from 4 sites), the average tissue Cd concentration was 1.21 ± 0.32 µg/g and ranged from 0.66 µg/g at Tillamook (January 2005) to 2.04 µg/g at Netarts Bay (September 2004).

5.1.3 Washington

Cadmium concentrations in Pacific oysters throughout Washington State increased in a northward and seaward direction with the exception of consistently elevated levels along the length of Hood Canal. A sharp decline was observed at the north end of Hood Canal between Bangor and Thorndyke Bay, approximately 1 nautical mile south of Hood Canal Bridge. Cadmium concentrations in the initial oyster composites (n = 92) ranged from 0.44 µg/g at Squaxin Island to 2.5 µg/g at Dabob Bay with a mean and standard deviation of 1.24 ± 0.57 µg/g.

Data was grouped into five distinct geographic regions according to cadmium distribution: south Puget Sound (SPS), north Puget Sound (NPS), Hood Canal (HC), north Hood Canal (NHC) and coastal estuaries (CE). South Puget Sound (9 sites) extended from Eld Inlet to Quartermaster Harbor, north Puget Sound (9 sites) from Agate Pass to Drayton Harbor and west to Sequim Bay, Hood Canal (7 sites) from Belfair State Park to Bangor, north Hood Canal (3 sites) from Thorndyke Bay to Port Gamble, and coastal estuaries (3 sites) from Grays Harbor to Willapa Bay.

Cadmium concentrations (means and standard deviations) for the five geographic regions and their relationship to the proposed Codex 1 µg/g ML for cadmium in molluscan shellfish are displayed in Figure 4. Differences between regions are statistically significant in all cases (ANOVA, p = 0.0018) except between SPS and NHC and between NPS and CE. Fifty six percent (56%) of oyster composites exceeded the proposed 1 µg/g ML and 17% of composites exceeded Hong Kong's 2 µg/g import standard.

Oyster tissue was also tested for zinc since the two metals are often associated together in the environment and evidence suggests that a diet rich in zinc may offer considerable protection against cadmium accumulation in animal and human subjects (Sullivan et al., 1984). Cadmium concentrations were positively correlated with zinc concentrations in Pacific oysters (rs = 0.78, p = 0) (Figure 5). Zinc concentrations ranged from 47 µg/g (wet weight) at Squaxin Island to 240 µg/g at Bangor with a mean and standard deviation of 109 ± 43 ug/g.

When including all oyster composites tested for both the initial sampling effort in addition to all other tissue samples through Fall 2006 (n=158 at 31 sites), the average tissue Cd concentration was 1.28 ± 0.54 µg/g and ranged from 0.44 µg/g at Squaxin Island (September 2004) to 3.19 µg/g at Hamma Hamma (April 2006).

5.1.4 Alaska

A total of six composites were collected from two Alaska sites during the initial Fall 2004 sampling effort. Cd concentrations ranged from 1.64 µg/g at Windy Bay to 2.49 µg/g also at Windy Bay with a mean and standard deviation of 2.03 ± 0.30 µg/g.

When including all oyster composites tested for both the initial sampling effort and all additional tissue samples through Fall 2006 (n=21 from 3 sites), the average tissue Cd concentration was 2.32 ± 0.50 µg/g and ranged from 1.64 µg/g at Windy Bay (September 2004) to 3.98 µg/g at Eaget Bay.

5.2 SEASONAL VARIATION OF CD IN SHELLFISH TISSUE

Pacific oyster composites were sampled between Fall 2004 and Fall 2005 (Winter 2006 at California locations) at a total of 10 locations: 2 in California, 2 in Oregon, 5 in Washington and 1 in Alaska. Seasonal sampling occurred in September, January, April, July and October respectively. Figure 6 depicts mean seasonal variation in Cd concentration at 10 locations. Because seasonal variation in Cd concentration can vary between regions and even between sites, the testing of statistically significant seasonal differences was performed separately for California, Washington and Oregon, and Alaska.

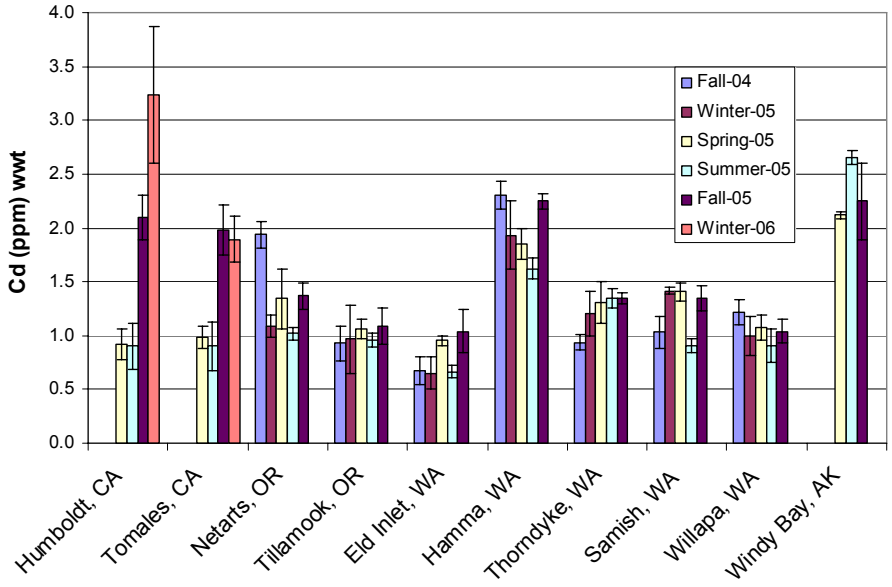


Figure 6. Seasonal variation in oyster Cd concentrations at 10 locations. Each bar represents the mean concentration derived from 3 adult oyster composites.

Fall 2004 data from the California locations was excluded due to an uncertainty of precise sampling location. In addition, seasonal sampling was not conducted at the California locations in Winter 2005 and was instead performed in Winter 2006. Seasonal data was collected at Chester Bay, AK in Fall 2004 and Spring 2005, but is not included in the seasonal variation figures and analyses due to the small size of the data set. Seasonal sampling was also determined to be unfeasible in winter months at the Alaska locations overall.

5.2.1 California

Samples were collected from Spring 2005 to Winter 2006 from Humboldt and Tomales Bays. Fall cadmium concentrations were significantly higher than Spring (t-test assuming equal variances, p=0) and Summer (t-test assuming equal variances, p=0) concentrations. Similarly, Winter concentrations were statistically higher than Spring (t-test assuming unequal variances, p=0.006) and Summer (t-test assuming unequal variances, p=0.003) concentrations. Fall and Winter concentrations were on average 148.2% higher than Spring and Summer.

5.2.2 Oregon and Washington

Seasonal samples were harvested from Netarts and Tillamook Bay in Oregon; and Eld Inlet, Hamma Hamma, Thorndyke, Samish Bay and Willapa Bay-Bay Center in Washington; from Fall 2004 to Fall 2005. Cadmium concentrations were statistically different seasonally (Friedman Test, p=0.05) with Fall 2005 concentrations being statistically higher than Winter 2005 (Wilcoxon Signed Rank Sum Test, p=0.043) and Summer 2005 (WSRST, p=0.018); and Spring 2005 being higher than Summer 2005 (paired t-test, p=0.013). On average, Spring and Fall concentrations were 17.6% and 27.8% higher than Summer respectively.

5.2.3 Alaska

Seasonal samples were collected in Spring, Summer and Fall of 2005 from Windy Bay, Alaska. Unlike California, Oregon and Washington, cadmium concentrations were statistically higher in Summer 2005 compared with Spring 2005 (t-test assuming equal variances, p=0.0002) by an average of 25.4%.

5.3 OYSTER TISSUE WEIGHT

Oyster tissue weights were measured at AmTest Laboratories for all Pacific oyster composites sampled between Fall 2004 and Fall 2005 (Winter 2006 at California locations). To calculate tissue weight per oyster, the total weight of each composite (shucked tissue + liqueur) was divided by the number of oysters per composite.

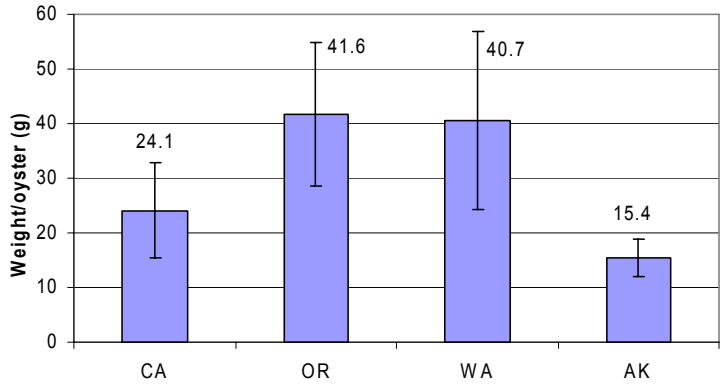


Figure 7. Mean shucked tissue weights per oyster for 4 geographic regions.

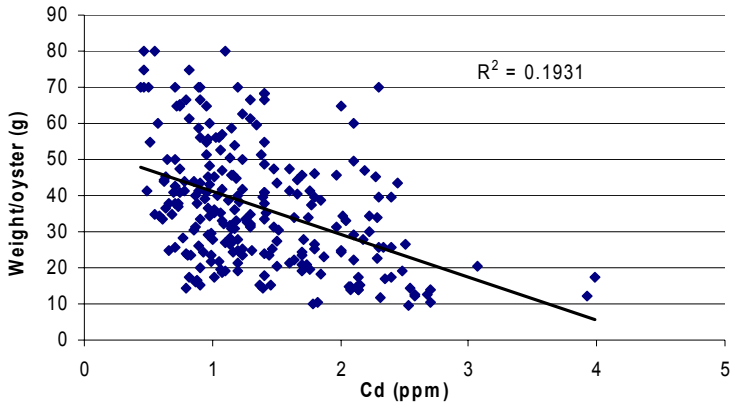


Figure 8. Relationship between adult tissue Cd concentration (ppm) and weight per shucked oyster (g) for all states (n=239).

Mean shucked tissue weights for all composites (n=239) at all station locations were 36.72 ± 16.69 grams per oyster (g/oyster) and ranged from 9.56 g at Annette Island, AK to 80 g at Dungeness Bay, WA. Mean tissue weights per oyster are displayed in Figure 7 for California (n=27), Oregon (n=39), Washington (n=152) and Alaska (n=21).

A statistically significant negative relationship was observed between tissue weight per oyster and tissue cadmium concentration for all states combined (rs = -0.429, p=0, R^2 = 0.1931) (Figure 8). The relationship between Cd concentration and weight per oyster varied, however, between different regions. The R^2 value was higher for the south Puget Sound region (R^2=0.3535) and lower for Hood Canal (R^2=0.0018). In Figure 9 the relationship between Cd concentration and oyster weight from regions with faster growth rates (Eld Inlet and Samish Bay) and slower growth rates (Hamma Hamma and Thorndyke Bay) within Washington are depicted. In faster growth rate areas, Cd levels decreased with increasing weight (r=-0.692, p=0.0002). In areas with slower growth rates, Cd levels appeared to increase with increasing weight, however the difference was not statistically significant. (r=0.2634, p=0.1842).

Table 1 depicts correlation data (Pearson Coefficient and Spearman Rank) derived from initial comprehensive sampling in September 2004, seasonal tissue sampling and two seed experiments (refer to Section 5.7.1– Growth Rates). Correlation analysis was performed for all states both considered together and individually. In addition, Washington data was analyzed separately for Hood Canal (HC), Coastal Estuaries (CE) and south Puget Sound (SPS).

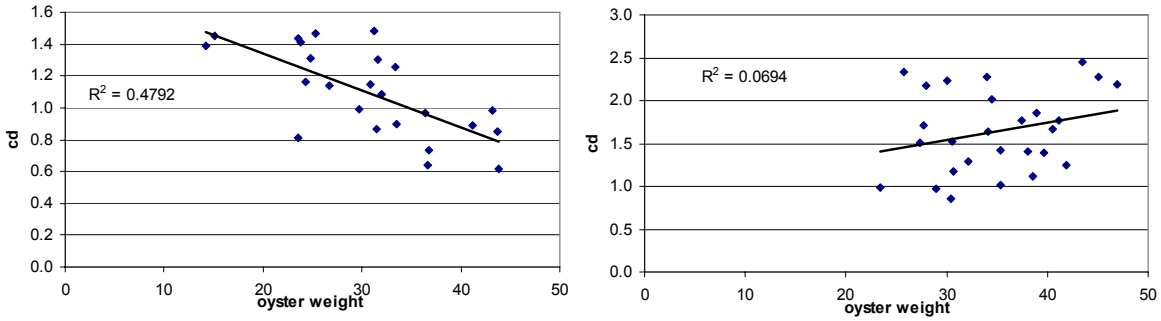


Figure 9. Relationship between Cd concentrations (ppm) and oyster weights at Eld Inlet and Samish Bay (left) and Hamma Hamma and Thorndyke Bay (right).

Table 1. Pearson Coefficient and Spearman Rank correlations derived from initial comprehensive sampling in September 2004, seasonal tissue sampling and two seed experiments. Underlined figures are significant to P<0.05.

Test condition	SD exp #1	SD exp #2	WA	HC	CE	SPS	CA	OR	AK	All
Cd level & oyster weight	-0.264	-0.464	-0.252	-0.043	-0.045	-0.658	-0.368	-0.260	-0.364	-0.429
Cd load & oyster weight	0.876	0.947	0.530	0.908	0.846	0.723	0.311	0.741	0.748	0.578
Cd level & Cd load	0.112	-0.234	0.423	0.363	0.458	-0.002	0.761	0.279	0.051	0.318
Cd load & oyster length	0.418	0.530								
Oyster length & weight	0.693	0.971								

5.4 SEASONAL VARIATION IN OYSTER TISSUE WEIGHT

Mean oyster tissue weights for each of the Pacific oyster composites sampled between Fall 2004 and Fall 2005 (Winter 2006 for California locations) at the 10 seasonal sampling stations are displayed in Figure 10.

5.4.1 California

Oyster composites were collected from Spring 2005 to Winter 2006 from Humboldt and Tomales Bays. Seasonal differences in adult shucked tissue weights per oyster were significant (ANOVA-single factor, p=0.0019) with Summer and Fall weights being statistically higher than both Spring and Winter (Tukey's multiple comparison method) by an average of 61.8%.

5.4.2 Oregon and Washington

Oyster composites were sampled seasonally from Netarts and Tillamook Bay in Oregon; and Eld Inlet, Hamma Hamma, Thorndyke, Samish Bay and Willapa Bay-Bay Center in Washington; from Fall 2004 to Fall 2005. Shucked tissue weights per oyster were significantly higher in Summer 2005 than Fall 2005 (p=0.02, paired t-test) by an average of 14.5%. Tissue weights were almost significantly higher in Summer 2005 than Spring (p=0.08, paired t-test) and Winter 2005 (p=0.07, paired t-test) as well.

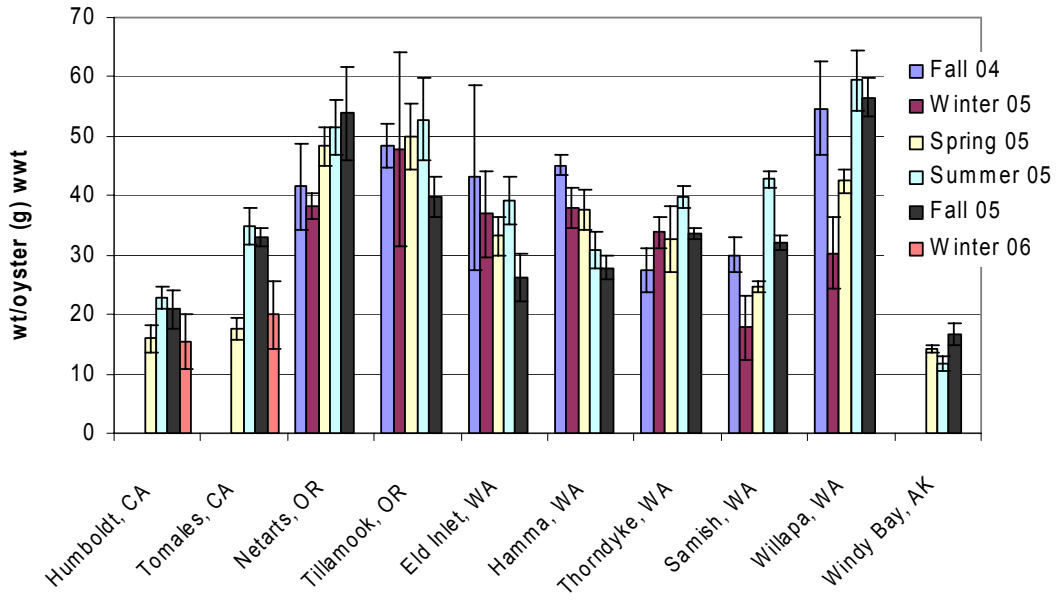


Figure 10. Seasonal variation in adult shucked tissue weights/oyster at 10 locations.

5.4.3 Alaska

Seasonal samples were collected in Spring, Summer and Fall 2005 from Windy Bay, Alaska. Oyster weights were statistically different seasonally ($p = 0.01$, ANOVA) with Summer 2005 weights being significantly lower than Fall weights by an average of 40%.

5.5 SEASONAL VARIATION IN SEDIMENT

Sediment samples were analyzed for metals (Cd and Zn), grain size analysis and total organic carbon at 10 locations from January 2005 to October 2005 (October 2006 for California locations). In October 2005, no sediment samples were collected from Humboldt, CA; nor was Zn tested at the Willapa Bay location. Sediment was collected and tested at four Washington locations in January 2006 while concurrently sampling oyster tissue for culture technique experiments. This data is not included in the following analyses, because with the exception of one location, the sediments were sampled from slightly different locations within each Bay or Inlet.

5.5.1 Metals

5.5.1.1 Cadmium

Cadmium concentrations in sediments ranged from undetectable levels at many of the locations to 3.3 mg/kg (dry weight) at Netarts Bay, Oregon in January 2005 (Figure 11). Overall, Cd concentrations were elevated at all locations during Winter 2005 and 2006. For the California locations, Summer 2005 was also slightly elevated. Oregon and Washington sampling locations showed similar trends with the highest sediment Cd concentrations in Winter and to a lesser degree in the Fall, and with almost undetectable levels in Spring and Summer. The Alaska location had the highest concentrations in Spring 2005, but sediments were not tested in Winter 2005 or 2006.

No relationship was found between sediment Cd concentrations and oyster tissue Cd concentrations ($r_s=0.07$, $p=0.66$).

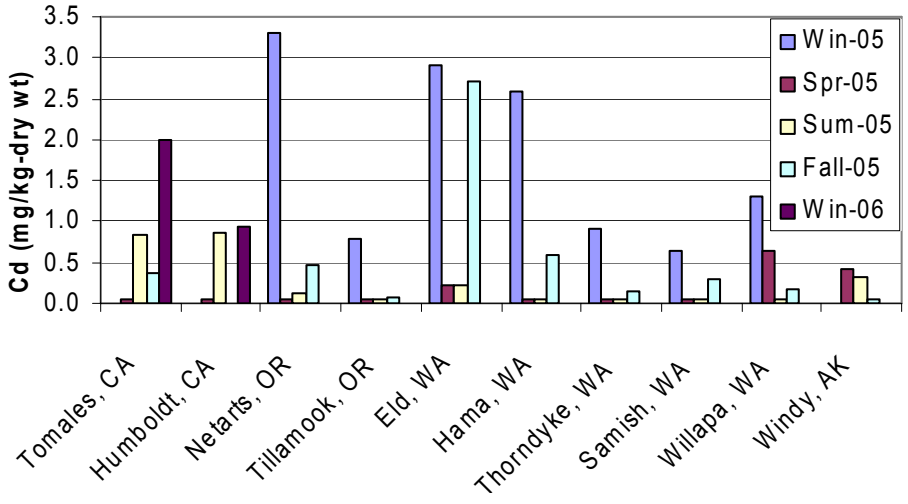


Figure 11. Seasonal sediment Cd concentrations (mg/kg-dry weight) from 10 locations. The detection limit ranged from .04 - .05 mg/kg.

Sediment cadmium concentrations fell below WDOE's Sediment Quality Standards (WDOE, 1993), NOAA's Sediment Quality Guidelines for Effects Range-Median (ERM) (NOAA, 1999) and Environment Canada's Environmental Quality Guidelines for Marine environment (EC, 2003) (Table X). Cadmium concentrations exceeded NOAA's Effects Range-Low (ERL) standard (1.2 mg/kg) as well as Environment Canada's Marine Interim Sediment Quality Guidelines (ISQG) (0.7 mg/kg).

5.5.1.2 Zinc

Sediment zinc concentrations ranged from 8.6 mg/kg at Samish Bay, WA (July 2005) to 110 mg/kg at Netarts Bay, OR (January 2005) (Figure 12).

At the California locations, zinc concentrations were the highest in Spring 2005. At the Oregon and Washington locations, zinc concentrations followed trends similar to cadmium with elevated levels during Winter 2005 and to a lesser degree Fall 2005. Alaska sediments had the highest zinc concentrations in Fall 2005, but were not tested in Winter 2005 or 2006. A positive relationship was observed between sediment Cd concentrations and sediment Zn concentrations (rs=0.44, p=0.008).

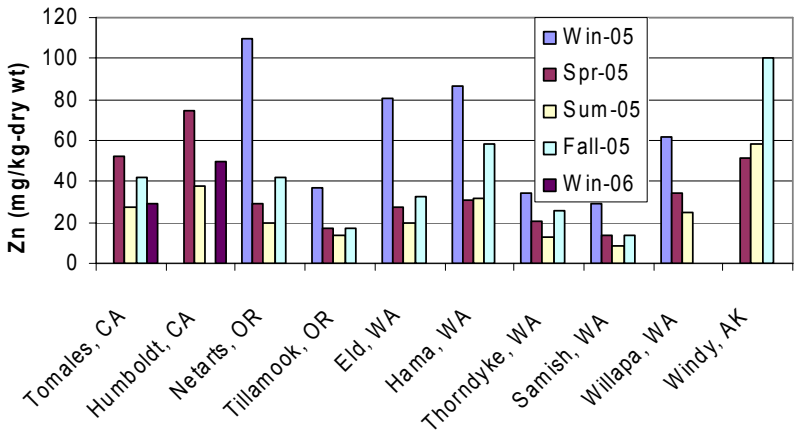


Figure 12. Sediment zinc concentrations (mg/kg-dry weight) from 10 locations. The detection limit ranged from .01 - .1 mg/kg.

5.5.2 Total Organic Carbon

Total organic carbon ranged from 0.1% at Thorndyke, WA (Winter 2005) to 3.3% at Eld Inlet, WA (Fall 2005) (Figure 13). No relationship was observed between total organic carbon and oyster tissue Cd concentration (rs=-0.23, p=0.17).

5.5.3 Grain Size Analysis

Sediment grain size distribution data portrayed unique profiles at each of the 10 locations. At each specific location, however, the profile was strikingly similar from season to season (Winter 2005 – Winter 2006). As a result, the following grain size distribution profiles are only representative of the Summer 2005 seasonal sampling event (Figure 14).

5.6 SEASONAL VARIATION IN SURFACE WATER

Seawater samples were analyzed for total and dissolved metals (Cd and Zn), total suspended solids (TSS) and Cd in seston from Winter 2005 to Fall 2005 (Winter 2006 for California) at 10 locations. Fall 2005 seawater samples were sent to Brooks Rand LLC for trace metal and TSS analysis.

5.6.1 Total and Dissolved Cadmium

Total Cd in seawater ranged from undetectable levels (0.02 µg/g) to 1.3 µg/g at Windy Bay, AK in Spring 2005 (Figure 16). Sixty percent of the sites had the highest concentrations during Summer 2005. The DL was 0.02 µg/g for Spring 2005, Fall 2005 and Winter 2006 and 0.006 µg/g for Fall 2005. No relationship was observed between total Cd in seawater and tissue Cd concentration (rs=-0.22, p=0.17).

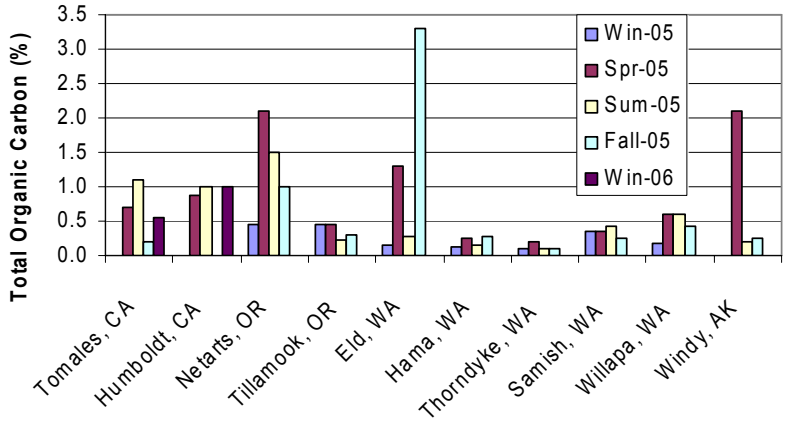


Figure 13. Total Organic Carbon (%) from 10 locations with a detection limit of .05%.

Dissolved Cd in seawater ranged from undetectable levels (0.02 µg/g) to 0.88 µg/g at Windy Bay, AK in Spring 2005(Figure 15). All Winter 2005 data was undetectable at the 0.5 µg/g detection level (DL) and was recorded as ½ the DL, or 0.25 µg/g , according to reporting methods described in Pyron's Data Validation Report (Appendix D).

The DL was 0.006 µg/g for Fall 2005 and 0.02 µg/g for the remainder of the seasons. Correlation analysis was not performed for dissolved Cd levels in seawater due to the large proportion of data that was undetectable at the 0.5 µg/g DL.

Water cadmium concentrations all fell below the EPA's Drinking Water Guidelines and National Recommended Water Quality Criteria for saltwater (USEPA 2004). Cadmium levels were also below the Environment Canada's Environmental Quality Guidelines (EC 2003) for the Maximum Acceptable Concentration (MAC) standard, but exceeded the Aquatic Life-Marine standard (0.12 µg/g) 31% (12 out of 39 samples) of the time for total Cd.

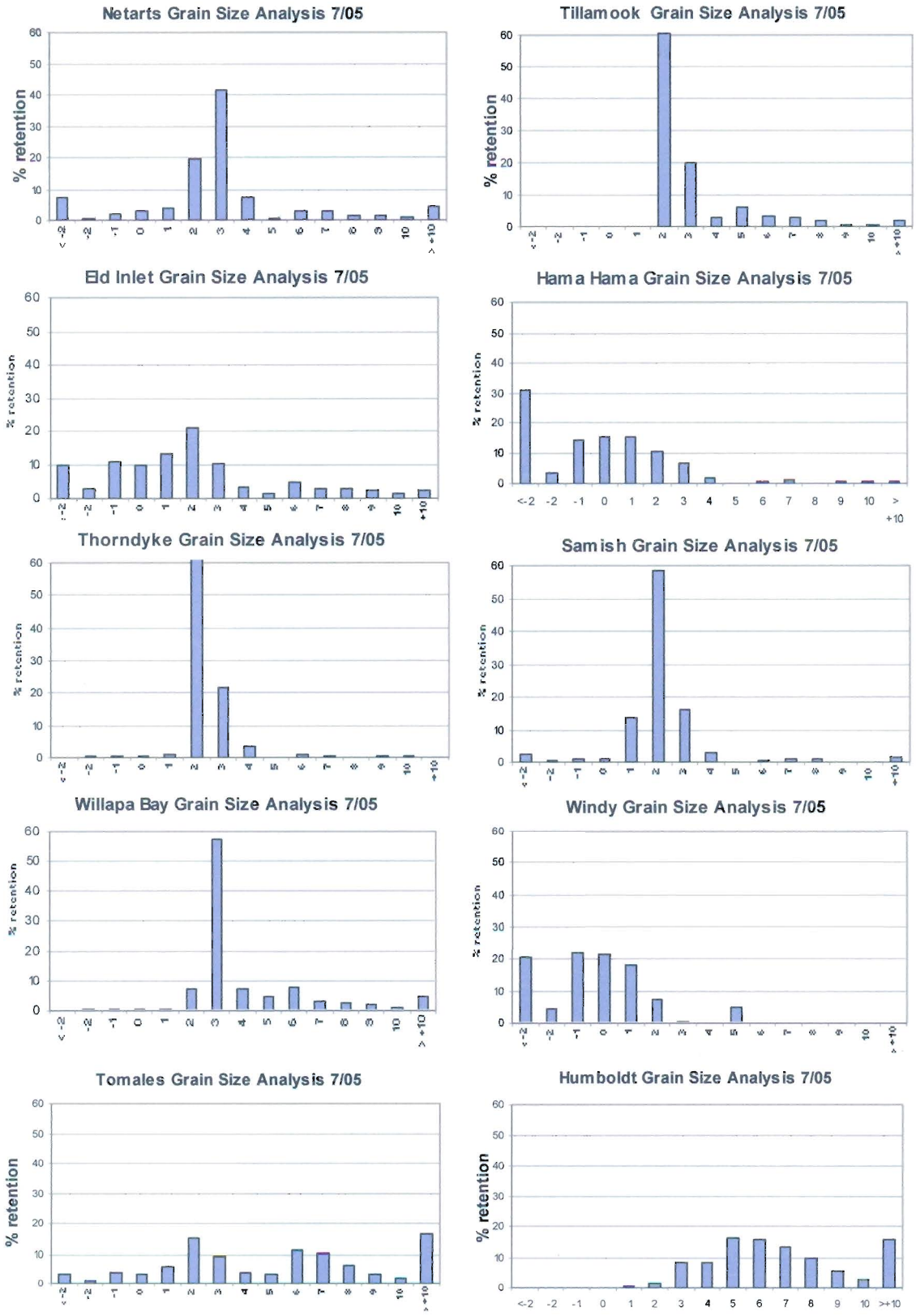


Figure 14. Grain size distribution (% retention) for 10 locations during Summer 2005 sampling event. Distribution represents gravel (-2), very coarse to very fine sand (1 – 4), coarse to very fine silt (5 – 8) and clay (9 – 10).

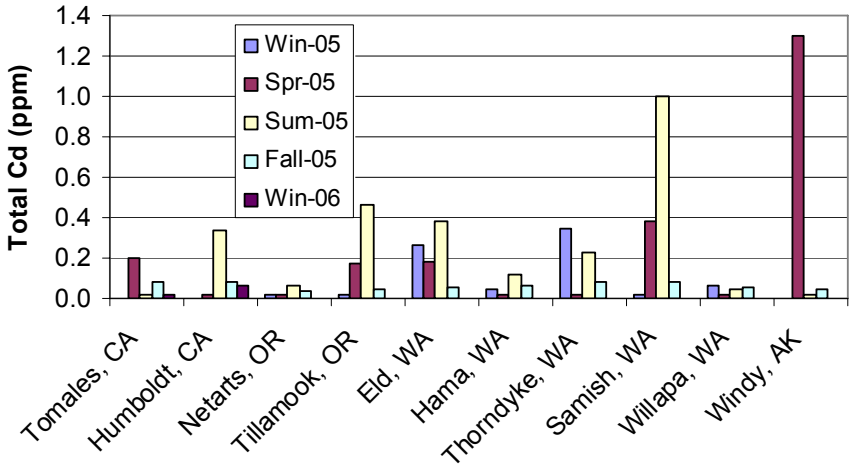


Figure 16. Total Cd in Seawater (ppm). The DL was 0.02 ppm for all data except Fall 2005 which was analyzed by Brooks Rand at the 0.006 ppm DL.

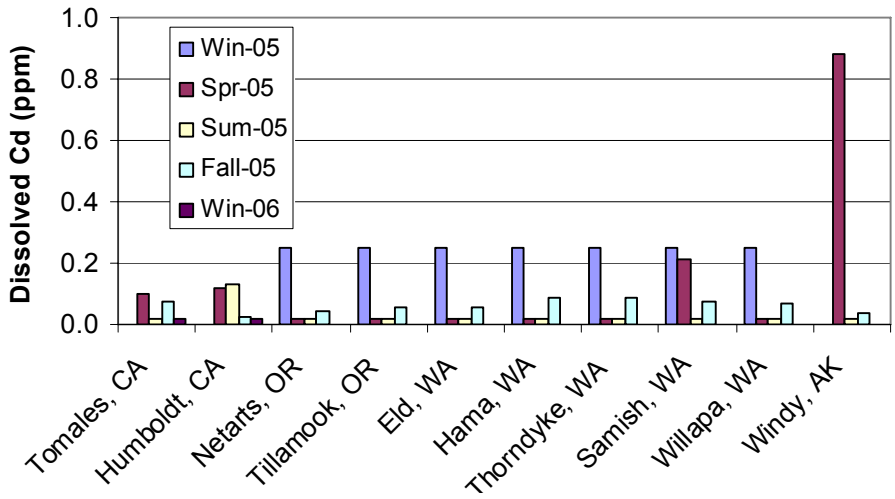


Figure 15. Dissolved Cd in Seawater (ppm). The DL was 0.25 ppm in Winter 2005, 0.006 ppm for Fall 2005 and 0.02 ppm for the remainder of the seasons.

5.6.2 Total and Dissolved Zinc

Total Zn in seawater ranged from 0.37 µg/g at Tomales Bay, CA in Fall 2005 to 400 µg/g at Humboldt Bay in Winter 2006 (Figure 17). Total Zn concentrations were consistently low during all sampling events with the exception of Humboldt and Tomales Bays in Winter 2006 and the Hamma location in Spring 2005. No relationship was observed between total Zn in seawater and tissue Zn concentration (rs=0.16, p=0.34), nor between total Cd in seawater and total Zn in seawater (rs=-0.26, p=0.11). Dissolved Zn in seawater ranged from undetectable at 0.095 µg/g (1/2 DL of 0.19 µg/g) at Tomales Bay in Fall 2005 to 380 µg/g at Humboldt Bay in Winter 2006 (Figure 17). Once more, notable spikes in dissolved Zn appeared at Humboldt and Tomales Bays in Winter 2006 and the Hamma location in Spring 2005. No relationship was observed between dissolved Zn in seawater and tissue Zn concentration (rs=0.27, p=0.10).

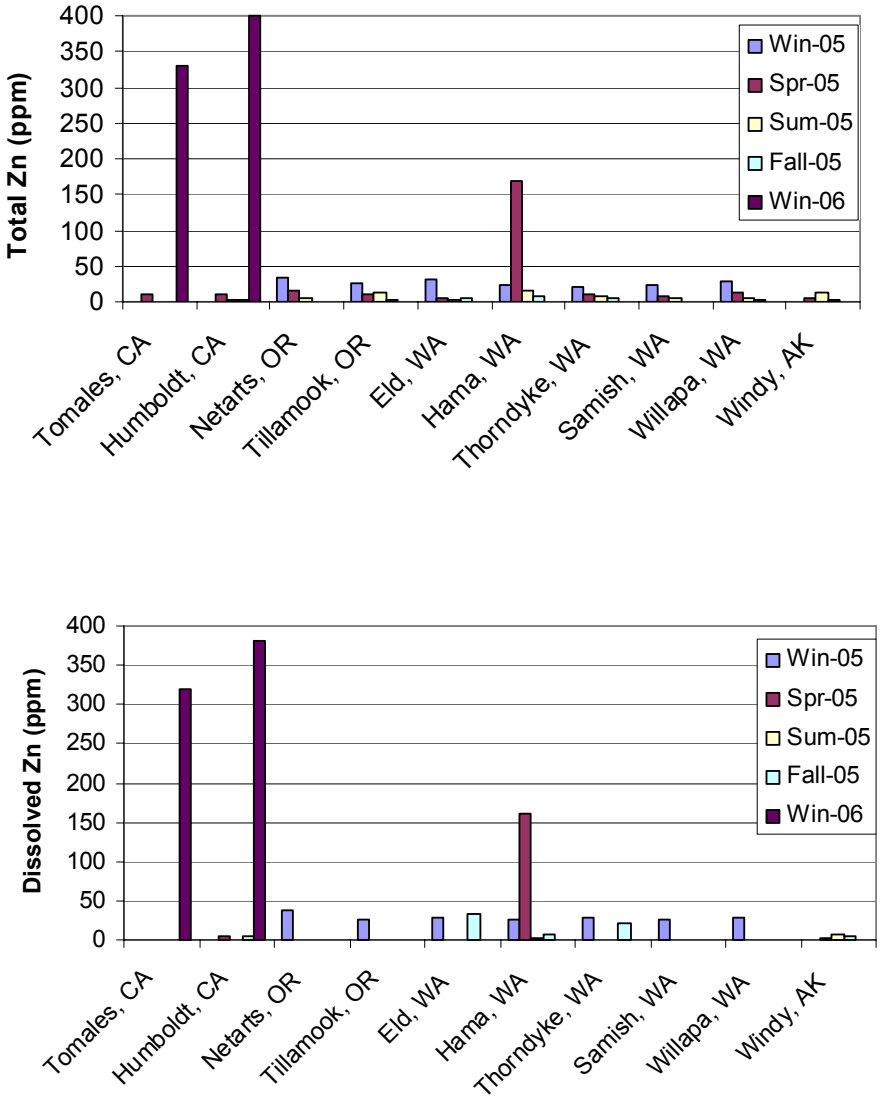


Figure 17. Total (top) and dissolved (bottom) Zn in Seawater (ppm). The DL was 0.19 ppm for Fall 2005 and 1 ppm for all other seasons.

5.6.3 Total Suspended Solids and Cd in Seston

Total suspended solids (TSS) ranged from undetectable (0.5 mg/l) at the 1 mg/l detection limit to 150 mg/l at Netarts Bay in Winter 2005 (Figure 19). Several spikes in TSS appeared in Winter 2005 and to a lesser degree in Spring 2005. No relationship was observed between TSS and tissue Cd concentration (r_s=0.02, p=0.88).

Cadmium concentrations in seston ranged from 0.000133 mg/kg at Netarts Bay in Winter 2005 to 0.66 mg/kg at Eld Inlet in Summer 2005 (Figure 18). All Fall 2005 data analyzed by Brooks Rand, LLC was undetectable at a DL ranging from 0.2 to 0.71 mg/kg (dry weight basis) except for Tomales Bay. Undetectable data (without qualifiers) were reported at ½ the DL as instructed in Pyron's Data Validation Report (Appendix D). AmTest results were converted to mg/kg (dry weight basis) by dividing the seston data (mg/l) by TSS (mg/l) and multiplying by 1000 (mg/kg). Correlation analysis was not conducted for Cd in seston levels due to the large proportion of data that was undetectable, particularly in Fall 2005.

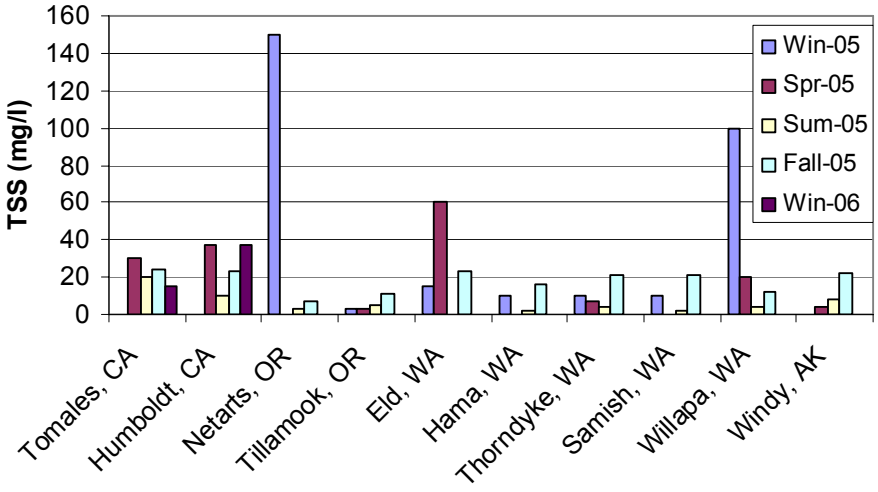


Figure 19. Total suspended solids (mg/l).

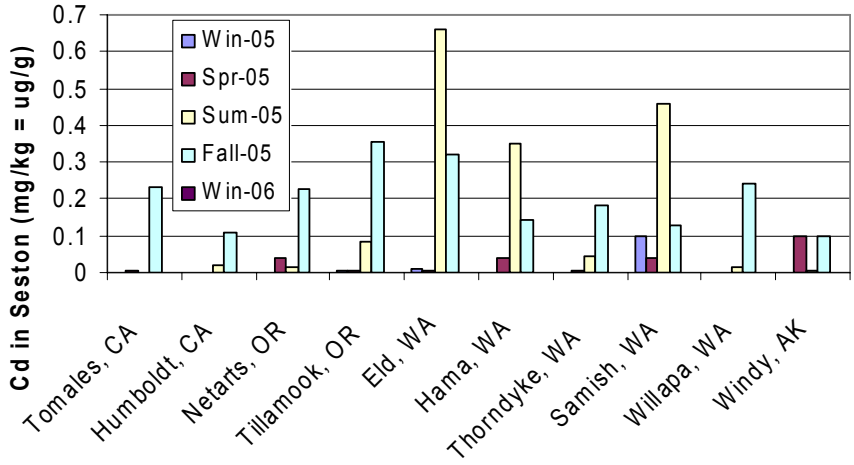


Figure 18. Cd concentration in seston (mg/kg-dry weight basis).

5.6.4 Phytoplankton

Phytoplankton samples were collected between Winter 2005 and Spring 2006 at 11 locations (Figure 20). Chester Bay, AK, was only sampled once, however, during Spring 2005. No sampling was performed at the California and Alaska locations in Winter 2005, nor were samples collected at Tomales Bay in Spring 2005. California samples were instead sampled in Winter 2006. Samples were only collected at five locations in Spring 2006. Phytoplankton composition and concentration often fluctuates rapidly making overall assumptions regarding seasonal variation based on one sample per season extremely difficult. For this reason, comprehensive data sets are presented in the following analyses to offer a more realistic account of seasonal variability and species composition for two locations in Washington State: Eld Inlet and Hood Canal. Phytoplankton abundance and speciation data was collected by Pacific Shellfish Institute from spring to fall at Eld Inlet (2002-2004) through funding from the National

Sea Grant's Oyster Disease Program (ODP), and year round at Hood Canal (2005-2007) for the Hood Canal Dissolved Oxygen Program – Integrated Assessment and Modeling Study.

5.6.4.1 Overall Seasonal Trend

In the Pacific Northwest, phytoplankton are typically found in low concentrations during the winter, followed by a spring bloom of predominantly centric, chain forming diatoms, that tapers off throughout the summer as nutrient levels are depleted. Dinoflagellates become more common at this time of the year since they are able to migrate vertically to gain access to nutrients found at depth. A second, smaller bloom typically appears in the fall and tapers off during winter months. The aforementioned trend was observed at Netarts Bay, Eld Inlet, and Willapa Bay. Of the largest bloom events (> 1 million cells/L), 50% occurred during the spring, 33% during the summer and 17% during the winter. A large amount of seasonal variation was observed not only between states, but also individual locations within states (Figure 20).

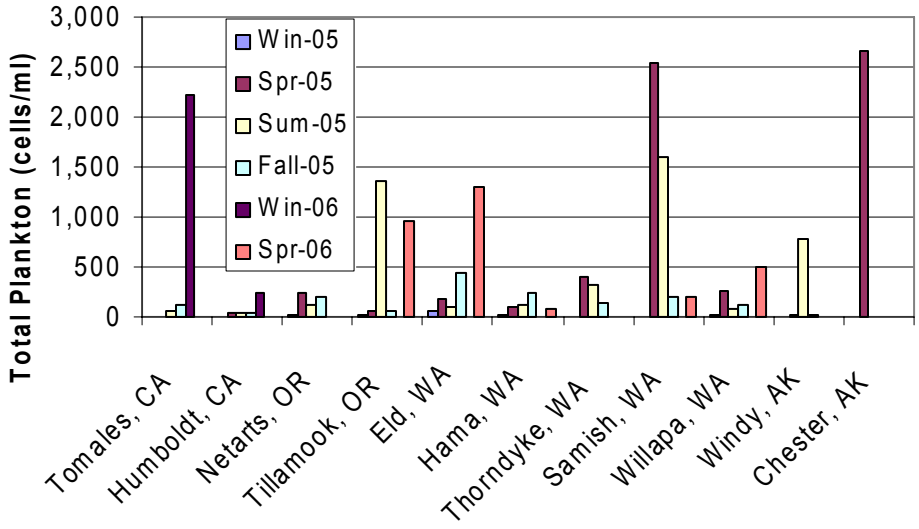


Figure 20. Seasonal variation in total plankton (cells/ml) at 11 locations. Counts include diatoms, dinoflagellates, flagellates (larger *Chromophyta*), and zooplankton.

5.6.4.2 Bloom Events

Six bloom events exceeding 1 million cells/L were observed between Winter 2005 and Spring 2006. The largest bloom was observed at Chester, AK in Spring 2005. Cell counts were greater than 2.6 million cells/L and consisted mostly of *Chaetoceros spp*. (1.6 million cells/L), *Skeletonema costatum* (398 cells/ml) and *Thalassiosira spp*. (492 cells/mL). Two blooms occurred at Samish Bay in Spring and Summer 2005 (2.54 million cells/L and 1.6 million cells/L). Dominant species during the bloom events at Samish were *Chaetoceros spp*. (1.98 million cells/L) and *Skeletonema costatum* (383 cells/ml) in the spring and *Eucampia zodiacus* (1.52 million cells/L) in the summer. One bloom occurred at Eld Inlet in Spring 2006 (1.2 million cells/L). Dominant bloom species included *Cerataulina pelagica* (838 cells/mL) and *Chaetoceros spp.* (352 cells/mL). One bloom was observed at Tomales Bay in Winter 2006 (2.23 million cells/L) and consisted primarily of *Skeletonema costatum* (1.35 million cells/L) and *Thalassiosira spp*. (662 cells/mL). Finally, a bloom was also observed in Tillamook, OR in Summer 2005 (1.36 million cells/L) consisting of mostly euglenoids (1.17 million cells/L). With the exception of euglenoids, a marine flagellate, all bloom species were chain forming centric diatoms.

5.6.4.3 Summary by Site

Phytoplankton samples were collected at Tomales Bay, CA from Summer 2005 to Winter 2006. Counts remained very low with the exception of a large bloom event in Winter 2006 (2.23 million cells/L).

Samples collected from Humboldt Bay, CA were also very low from Spring 2005 to Fall 2005. In Winter 2006, counts increased to 242 cells/mL and consisted predominantly of a mix of *Thalassiosira spp*. (62 cells/mL) and *Cylindrotheca closterium* (46 cells/mL).

Netarts Bay, OR exhibited seasonal trends more typical of the Pacific Northwest with low concentrations in the winter followed by elevated cell counts in the spring (244 cells/mL) that tapered off during summer months and increased once more, but to a lesser degree in the fall. During Spring 2005, *Pseudo-nitzschia* (192 cells/mL), a genus that contains several species that produce the neurotoxin domoic acid, was observed.

Cell counts remained low at Tillamook, OR in Winter, Spring and Fall 2005. A large bloom occurred during Summer 2005 (1.36 million cells/L) consisting of mostly euglenoids (1.17 million cells/L) and again in Spring 2006 (952 cells/mL) consisting of predominantly *Chaetoceros spp., Thalassiosira spp.* and *Asterionellopsis socialis*.

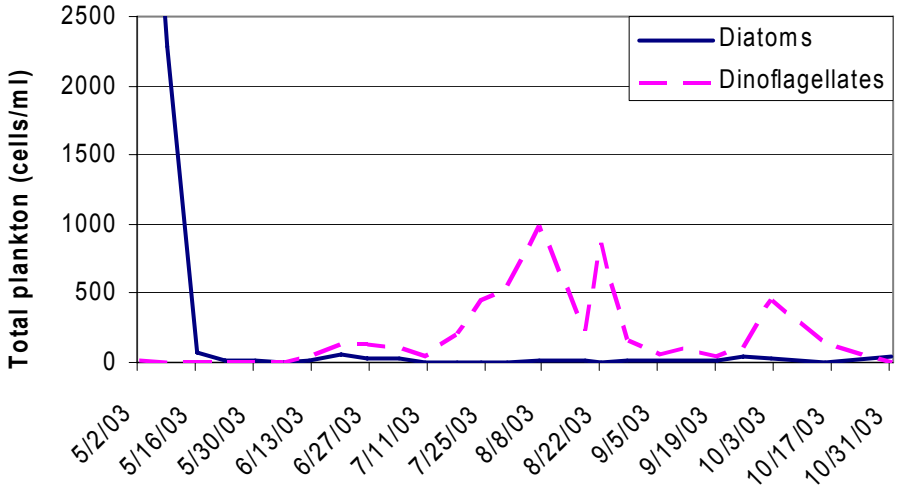


Figure 21. 2003 Eld Inlet weekly plankton counts (cells/ml) collected during the National Sea Grant's Oyster Disease Program research project.

At Eld Inlet, WA, plankton counts were low during Winter 2005. Ciliates (82 cells/mL) appeared in the spring followed by the appearance of dinoflagellates (predominantly *Ceratium fusus*) in the summer. A significant bloom of dinoflagellates (196 cells/mL) and ciliates (146 cells/mL) appeared in the fall followed by a bloom (1.3 million cells/L) of *Ceratualina pelagica* and *Chaetoceros spp*. in Spring 2006.

Plankton sampled during the Oyster Disease Project was collected weekly from Eld Inlet, WA from spring to fall (2002 – 2004) and showed a similar seasonal trend. In 2003, a large spring bloom (10.1 million cells/L) dominated primarily by *Cerataulina pelagica* and to a lesser extent *Chaetoceros spp*., both centric diatoms, tapered off during the summer giving way to a smaller bloom of *Ceratium fusus*, a dinoflagellate, in late summer and early fall. Maximum bloom concentrations remained below 2 million cells/L during 2002 and 2004 (Figure 21).

At the Hamma Hamma (Hama), WA location, counts were relatively low throughout the entire year. In Spring 2005, dinoflagellate counts (52 cells/mL) were higher at this location than any of the other nine sites for that time period and consisted of a mix of *Scrippsiella trochoidea* and unidentified dinoflagellates. Hamma cell counts were highest during Fall 2005 (244 cells/mL) and consisted of mostly *Chaetoceros spp*., *Actinoptychus senarius*, *Thalassiosira spp*., ciliates, and small dinoflagellates.

Plankton samples were collected weekly at 3 locations in southern Hood Canal from 2005 - 2007 as part of the Hood Canal Dissolved Oxygen Project (HCDOP). The Sisters Point and Potlach stations are located at the bend along the southern end of the Canal and Bambans Cove is located north of the bend and south of Lilliwaup. According to the impressions of several shellfish growers interviewed during the Economic Assessment Report, the southern end of the Canal near Sisters Point experiences very thick plankton concentrations and rapid shellfish growth while the region midway up the length of the Canal experiences sparse concentrations and slower shellfish growth. Data collected from the HCDOP supports these testimonies in that the southernmost site locations experienced tremendously elevated cell counts.

At Sisters Point and Potlach, spring blooms were observed in 2006 (as high as 69 million cells/L) and 2007 (>10 million cells/L) that were dominated primarily by *Chaetoceros spp*. and *Detonula pumila*. In late summer and early fall 2006, blooms consisted almost entirely of dinoflagellates and ranged from 10-44 million cells/L. Similar to Tomales Bay, the southern Hood Canal sites experienced blooms of centric diatoms (*Chaetoceros spp., Ditylum brightwellii*) throughout the winter months that ranged from 2.5-6 million cells/L.

Bambans Cove, located closest to the Hamma location, also experienced spring blooms of centric diatoms, but at concentrations ranging from 2-2.5 million cells/L. Similar to Sisters and Potlach, blooms consisting almost entirely of dinoflagellates and zooplankton (tintinnids) dominated samples in late summer and early fall. Blooms of centric diatoms continued to spike throughout the winter (2-4 million cells/L).

At Thorndyke Bay, WA, cell counts were low throughout most of the year. Plankton counts were highest for this site during Spring 2005 (401 cells/mL) and consisted of predominantly *Chaetoceros spp*. *Chaetoceros spp*. was also the main species encountered during the summer.

Samish Bay, WA experienced large blooms in both Spring and Summer 2005 (2.54 million cells/L and 1.6 million cells/L). Dominant species during the bloom events at Samish were *Chaetoceros spp*. (1.98 million cells/L) and *Skeletonema costatum* (383 cells/mL) was also observed in Spring 2006.

Willapa Bay, WA experienced a small spring bloom in 2005 (256 cells/ml) of mostly *Skeletonema costatum* and a larger spring bloom in 2006 (498 cells/ml) of mostly *Chaetoceros spp*. (156 cells/ml) and *Asterioniellopsis socialis* (118 cells/ml).

Windy Bay, AK had very low cell counts in Spring and Fall 2005. In Summer 2005, a spike was observed (784 cells/ml) consisting of mostly *Skeletonema costatum* (372 cells/ml). *Pseudo-nitzschia* (58 cells/ml) was also found in the sample.

Chester Bay, AK was only sampled once in Spring 2005. Cell concentrations were over 2.6 million cells/L and consisted mostly of *Chaetoceros spp*. (1.6 million cells/L), *Skeletonema costatum* (398 cells/ml) and *Thalassiosira spp*. (492 cells/ml).

5.6.5 Physical Oceanography (DOE water data – temp, salinity, pH)

Seawater temperature was lowest during winter months, increased throughout the summer and declined in the fall at WDOE water quality sampling stations (Figure 22). Temperature fluctuated more at the surface than at depth (10 m) with the greatest difference occurring during summer. The lowest and highest July surface temperatures (means and standard deviations) were 15.13 ± 1.54ºC and 18.89 ± 0.76ºC at Bangor and Hamma Hamma respectively. Peak surface temperatures were highest (>20 ºC) in July at Hamma Hamma and Toke Point.

Salinity was lowest during winter months and increased throughout the summer and into the fall at most stations (Figure 23). Salinities were lower, and fluctuated more, at the surface than at depth (10 m) and the difference between surface and depth measurements were greatest during winter and spring. In January, the lowest and highest salinities ranged from 21.19 ± 4.20 ppt (parts per-thousand) and 28.09 ± 1.68 ppt at Toke Point and Bellingham respectively. Surface salinities were measures as low as 15.06 ppt and 17.04 ppt at Toke Point and Hamma Hamma.

Seasonal pH measurements were generally low during Winter, increased in Spring, remained elevated throughout Summer and decreased in Fall (Figure 24). Two exceptions include Toke Point that demonstrated more seasonally fluctuating readings and Eld Inlet that exhibited elevated readings throughout Fall. Error bars represent large variability at most sites and during most seasons. Mean pH values ranged from 7.61 (Eld Inlet at depth) to 8.21 (Hamma Hamma at surface) with individual pH readings as low as 7.1 and as high as 8.8 in surface waters at Toke Point.

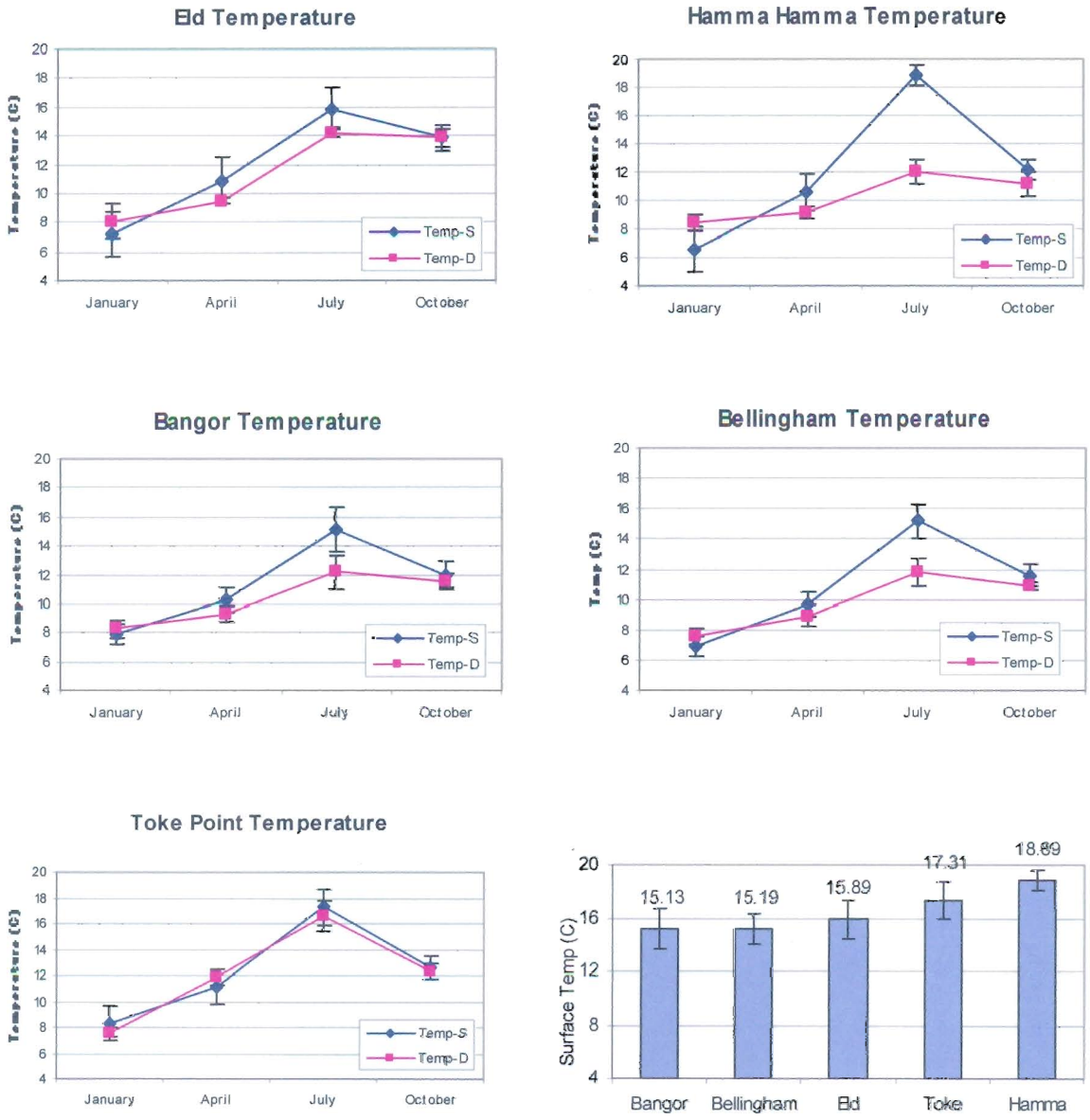


Figure 22. Seasonal seawater temperatures at surface and depth at five WDOE sampling stations. The bar chart represents mean July surface temperatures at each of the stations.

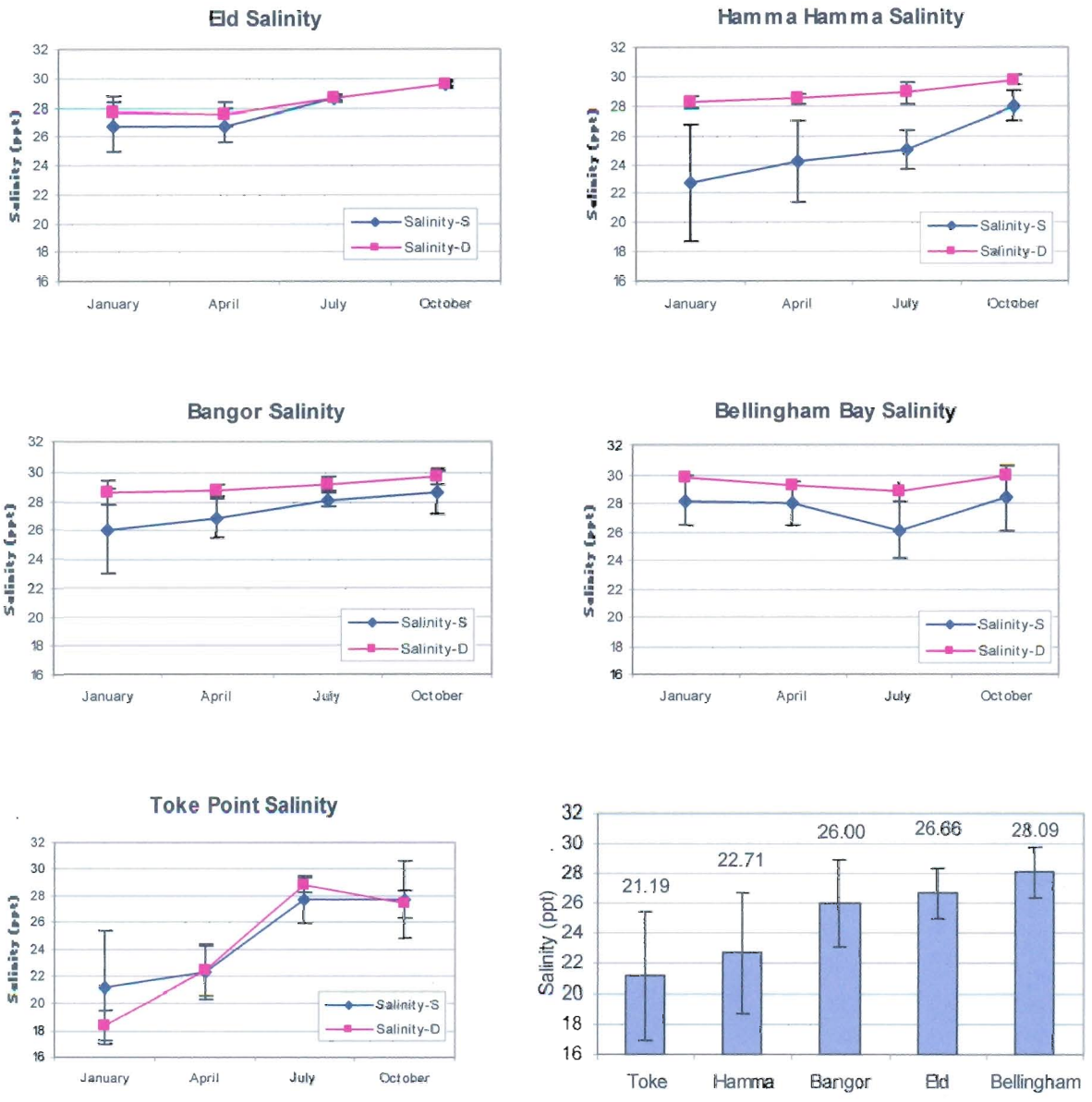
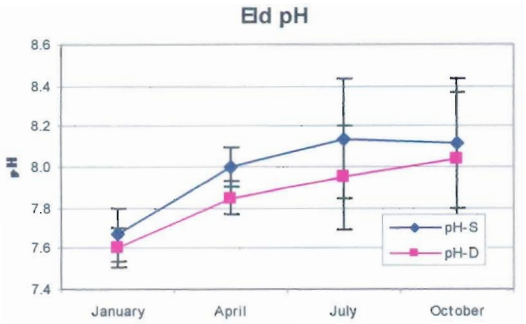
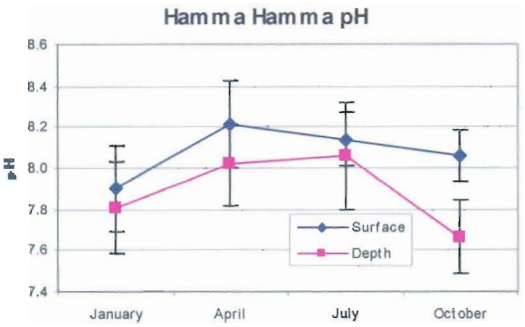
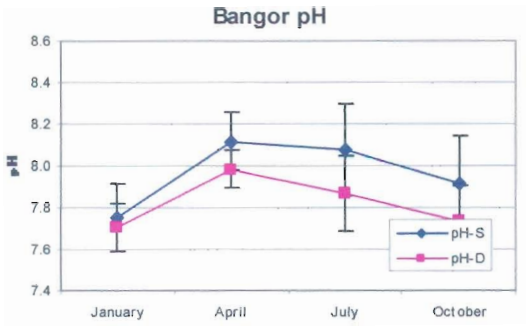
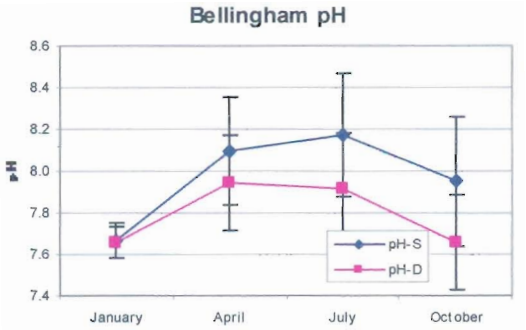


Figure 23. Seasonal seawater salinities at surface and depth from five WDOE sampling stations. The bar chart represents mean January surface salinities at each of the sampling stations.









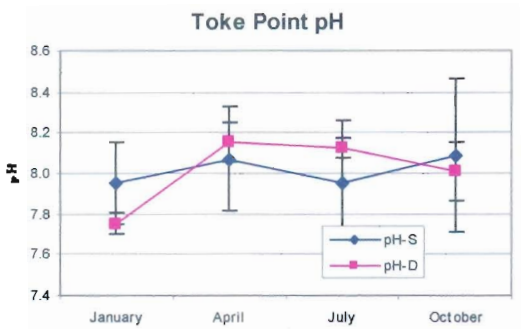


Figure 24. Seasonal pH at surface and depth at five WDOE sampling stations.

5.7 ADDITIONAL FACTORS

5.7.1 Growth Rates

Shell lengths (n=30/site) were measured on Day 1 (initial), Day 73 (July 2005) and Day 169 (October 2005) during a growth rate experiment conducted at five Washington locations (Figure 25). Significant differences were detected between the five sites (p=0, Kruskal-Wallis Test). By Day 169, growth rates at Thorndyke Bay were the slowest (0.18 mm/day). Seed at Samish Bay grew faster than Thorndyke (0.24 mm/day) (p=0.006, t-test assuming unequal variances) but was not statistically different than Hamma Hamma (.24 mm/day). Growth rates at Eld Inlet (0.30 mm/day) were almost faster than Hamma Hamma (p=0.08, Wilcoxon Rank Sum Test), but the difference was not significant. The fastest growth rates were observed at Willapa Bay (.32 mm/day). At Samish Bay, oyster drills caused considerable mortality in the larger sized seed which may have resulted in a lower-than-actual growth rate.

Testimonials from oyster growers in Eld Inlet and Hood Canal should also be considered when evaluating growth rates between regions. According to several growers, a 4-6 inch oyster can be grown in 2-3 years in Eld Inlet, whereas, it takes up to 5 years to reach this size in central Hood Canal. However, one shellfish grower acknowledged that it takes a couple of years to grow a 4-6 inch oyster in the southern portion of Hood Canal near Sister's Point due to high primary productivity in that area. In addition, the growth rate of seed oysters in Eld Inlet was reported to be atypically slow in the first few months and then increases substantially thereafter.

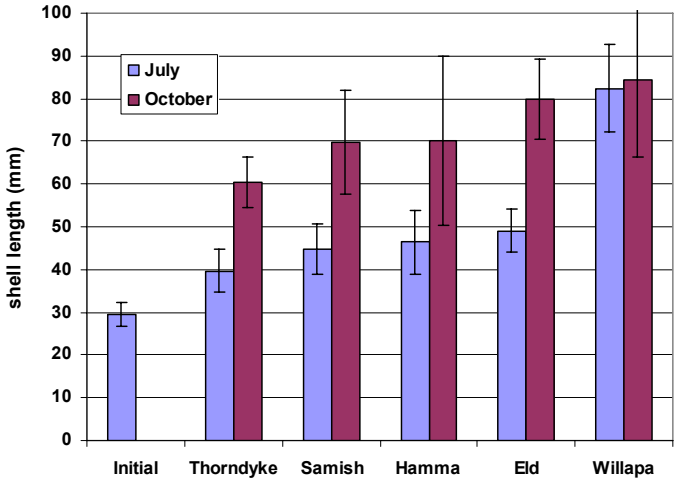


Figure 25. Average shell lengths (mm) during 169 day growth rate experiment at five locations.

Figure 26 displays the average unshucked weight per individual oyster seed (n=30/site) after 169 days at five Washington locations. Average weights ranged from 20 g at Hamma Hamma to 39 g at Eld Inlet. Individual oyster weights at Samish Bay were significantly higher than Hamma Hamma (p=0, t-test assuming equal variances) and Thorndyke Bay (p=0.002; t-test assuming unequal variances). Oyster weights at Eld Inlet were statistically higher than Samish Bay (p=0, t-test assuming equal variances).

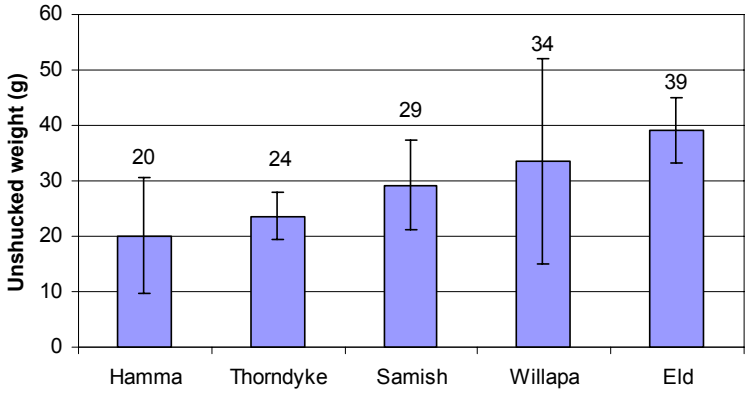


Figure 26. Average unshucked oyster seed weights (g) at five locations.

Oyster seed was also tested for Cd concentration on Day 1, Day 73 and Day 169 during the growth rate experiment (Figure 27). The initial seed Cd concentration on Day 1 was 1.6 µg/g. Average seed concentrations at Day 169 ranged from 0.9 µg/g at Willapa Bay to 1.6 µg/g at Hamma Hamma. When evaluating all five sites together, no significant difference in concentration was detected between July and October (p=0.69, Wilcoxon Signed Rank Sum Test). Only one oyster composite was tested at Samish Bay in October due to high seed mortality rate caused by oyster drills.

Diploid and triploid oyster seed was also placed at four Washington locations

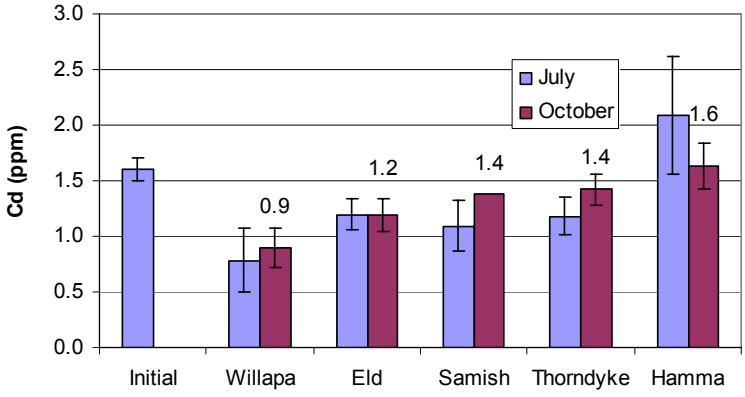


Figure 27. Oyster seed Cd concentrations at five Washington locations.

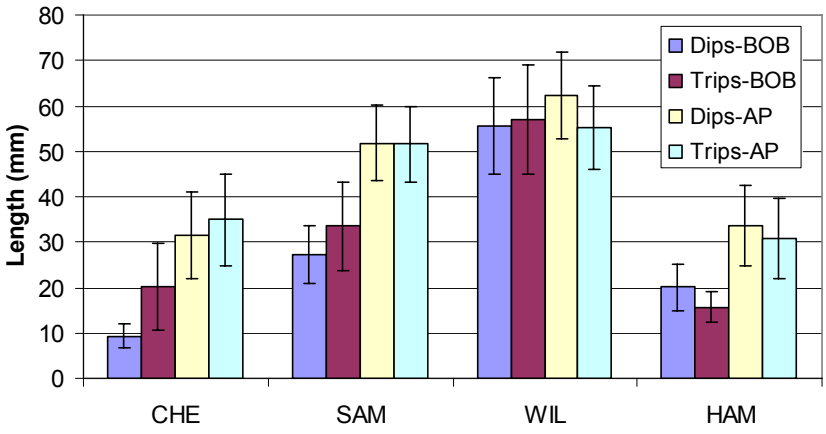


Figure 28. Average oyster seed lengths after 117 days at four Washington locations. Each bar represents the average length calculated from three composites of approximately 30 seed each.

(Chelsea Farms, Eld Inlet, Samish Bay, Willapa Bay, Hamma Hamma) for a 117 day period from April to August 2006. While the experiment was primarily conducted to evaluate the impact of culture technique (bag-on-bottom and aqua purses) and oyster ploidy on tissue Cd concentrations, individual oyster length data was recorded to further assess growth rates between various regions (Figure 28). Seed placed in oyster bags (BOB) and aqua purses (AP) at Eld Inlet (CHE), Samish Bay (SAM) and Willapa Bay (WIL) were in slightly different locations than for the initial growth rate experiment conducted in April and October 2005.

Differences in oyster seed lengths were detected between the four sites (p=0, Kruskal-Wallis Test). Oyster seed at Samish Bay almost grew faster than Eld Inlet (CHE), but the difference was not significant (p=0.0558, Wilcoxon Rank Sum Test). Growth rates at Samish Bay were slower than Willapa Bay (p=0, t-test assuming unequal variances) and faster than Hamma Hamma (p=0.001, t-test assuming equal variances). Seed lengths at Hamma Hamma were not significantly different than Eld Inlet.

5.7.2 Culture Technique and Oyster Ploidy

To test the impact of culture method and oyster ploidy on Cd concentration, diploid and triploid seed were placed at four Washington locations (Chelsea Farms, Eld Inlet, Samish Bay, Willapa Bay, Hamma Hamma) in adjacent on-bottom bags or off-bottom aqua-purses for a 117 day period between 4/27/06 and 8/21/06. In August 2006, a total of 12 oyster composites (3 per treatment) were collected and analyzed for tissue Cd concentrations at each site (Figure). Seed Cd concentrations were tested on April 27, prior to being placed in the field. Initial mean Cd concentrations (T-0) in diploid and triploid seed were 0.707 ± 0.176 µg/g and 0.927 ± 0.316 µg/g respectively. After 117 days, the mean Cd concentration in triploid seed grown in aquapurses at Hamma Hamma was 3.477 ± 0.294 µg/g.

5.7.2.1 Culture Technique

When comparing mean Cd concentrations across all four sites considered together, concentrations were significantly higher in seed grown in aquapurses (AP) than those grown using the bag-on-bottom (BOB) culture technique (p=0.028, Wilcoxon Signed Rank Sum Test). Similarly, when comparing all composites (not composite means) for each treatment at the four sites separately, oyster concentrations in aquapurses were significantly higher than on-bottom bags at Willapa Bay (p=0.013, t-test assuming equal variances) and Hamma Hamma (p=0.007, t-test assuming unequal variances). However, no significant difference was detected between seed grown in aquapurses and on-bottom bags at Eld Inlet (p=0.991, t-test assuming equal variances) and Samish Bay (p=0.788, t-test assuming unequal variances).

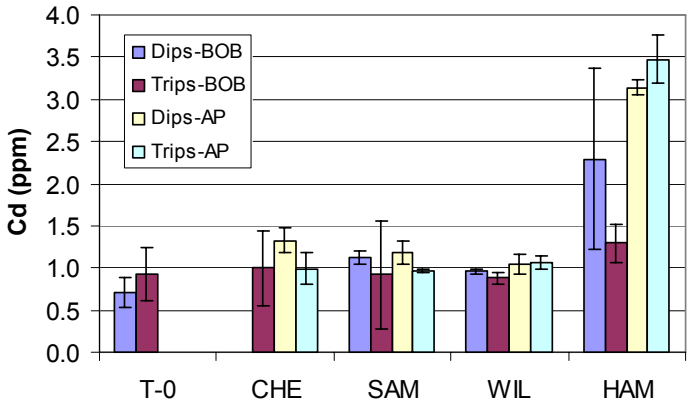


Figure 29. Mean tissue Cd concentrations for diploid and triploid oyster seed grown in aquapurses and on-bottom bags at four Washington locations.

Mean oyster lengths were significantly greater using aquapurses than on-bottom bags (p=0.002, paired t-test) when considering all four sites together. When evaluating each of the sites separately, seed lengths remained greater for aquapurses than on-bottom bags at Eld Inlet, Samish Bay and Hamma Hamma, however, the difference was not significant at Willapa Bay. Shucked weights per oyster were also statistically greater when grown using aquapurses than on-bottom bags (p=0.02, paired t-test) (Figure). Total Cd load reflects the amount of Cd that a consumer ingests per oyster (Figure 29). Total Cd loads were greater in oyster seed grown using aquapurses than on-bottom bags (p=0.018, Wilcoxon Signed Rank Sum Test). Despite the fact that oyster seed grown at Samish Bay and Willapa Bay had lower Cd concentrations, total Cd loads were relatively high, particularly for aquapurses, due to the larger weights per oyster. Similarly, while Cd concentrations were higher at Hamma Hamma, low weights per oyster resulted in much lower total Cd loads.

5.7.2.2 Oyster Ploidy

No significant differences were detected in Cd concentration (p=0.237, Wilcoxon Signed Rank Sum Test), weight per oyster (p=0.302, paired t-test), total Cd load (p=0.926, paired t-test) and oyster length (p=0.674, paired t-test) between diploid and triploid oyster seed when considering all sites together.

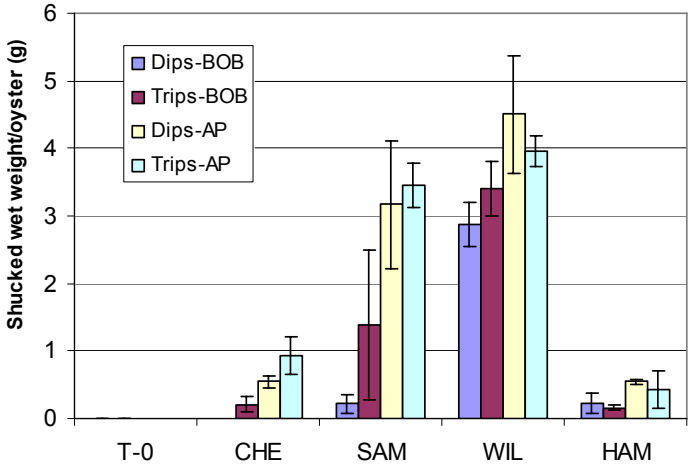


Figure 30 . Weight per shucked oyster for diploid and triploid seed using two culture techniques.

5.7.3 Processing

Cd Concentrations. Processing methods and storage did have an effect on the Cd concentrations in oyster tissue. Draining shucked oysters resulted in a significant increase ($p < 0.05$) in the Cd concentration, which rose from 1.1 to 1.7 mg/kg. This indicates that Cd is primarily concentrated within the oyster tissue and is not released with the nectar during shucking. Washing and jar-packing of shucked and drained oysters (Group S-WJ) led to a significant reduction in the Cd concentration, down to 1.4 mg/kg. These oysters showed gradual decreases in the Cd concentration during refrigerated storage, with levels of 1.1 and 0.9 mg/kg at days 5 and 10, respectively. The Cd concentration in oysters after 10 days of refrigerated storage was significantly lower than the concentration at day 0. The reductions observed in Cd concentration during washing and jar-packed storage of shucked and drained

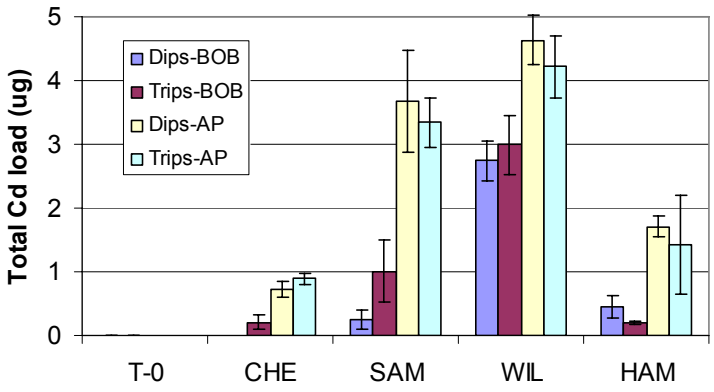


Figure 29. Total Cd load for diploid and triploid oyster seed using two culture techniques.

oysters could be a result of a release of Cd into surrounding water, an uptake of water by the oyster tissue, or a combination of both events. A release of Cd by the oyster tissue could be reflected by reductions in the total Cd present within the oyster tissues and uptake of water could be reflected by increases in oyster tissue weight.

Total Cd (µg/oyster). The total levels of Cd (µg) per oyster were somewhat influenced by processing and storage. As shown in Table 1 and Fig. 2, total Cd load increased following draining of shucked oysters, but the difference was not significant. This was due to high variability among the oysters in Group SD, which had total Cd loads ranging from 28.2 to 50.1 µg/oyster. Likewise, washing and jar-packing did not result in a significant difference in total Cd at day 0. This is different from the results found above, in which Cd concentration decreased significantly following washing and jar-packing. A case in which the concentration decreases but the overall Cd load does not suggests a difference in tissue weights (total Cd accounts for both tissue weight and Cd concentration). Both the initial and final tissue weights of oysters in Group S-WJ analyzed at day 0 were relatively high (Table 2), possibly resulting in a higher total Cd load. As in the case of Cd concentrations, there was a gradual decrease in Cd load in Group S-WJ during the 10-day storage period, with a significant difference between the total Cd at day 0 (36.7 µg/oyster) and day 10 (21.2 µg/oyster). However, it is not clear whether this is due to a release of Cd into the jar water or simply a result of the relatively heavy weight of the oysters that were analyzed at day 0.

Moisture content. Changes in the moisture content of oyster tissues during processing and storage may have influenced the Cd concentration in oysters by increasing or decreasing the dilution factor. Draining the nectar from the oysters may have concentrated the Cd within the tissues, while washing in an aerated water bath may have increased the moisture content and diluted the Cd present. Water uptake during jar-packed storage may have further diluted the Cd in tissues, contributing to decreases in Cd concentrations but not total Cd loads. Uptake of additional moisture during jar-packed storage can be measured by comparing the initial and final drained weights of the oysters (Table 2). Paired sample t-tests revealed significant increases in the weights of oysters in Group S-WJ at days 0, 5 and 10 compared to the initial weights. These weight increases are likely the result of increases in moisture load, which could have contributed to the observed decreases in Cd concentration following washing, jar-packing and storage.

Group HPP

Cd concentration. As shown in Table 1 and Fig. 1, the initial Cd concentration in oysters from Group HPP was 1.1 mg/kg, which was significantly lower than the initial concentrations for oysters from Group SD (1.7 mg/kg) and Group S-WJ (1.4 mg/kg). There were gradual decreases in the Cd concentrations in oysters in Group HPP at days 5 (0.9 mg/kg) and 10 (0.8 mg/kg) of storage; however, unlike Group S-WJ, none of the reductions were significant. These trends indicate that events may occur during the HPP treatment that cause a reduction in Cd levels, possibly due to a release of total Cd from the oyster tissue or an uptake of water.

Total Cd (µg/oyster). As shown in Table 1 and Fig. 2, oysters in Group HPP had significantly lower total Cd levels than oysters in Group S-WJ (day 0), with total Cd loads of 20.8 and 34.7 µg/oyster, respectively. The Cd loads in Group HPP oysters were also significantly lower than oysters in Group SD. These oysters had similar final weights (Table 2), implying that the lower Cd concentrations in HPP oysters may be due to a release of Cd from the oyster tissue, rather than water uptake. Despite the initial drop in Cd levels following HPP treatment, storage in water-filled jars over 10 days did not result in further decreases, and by day 5 of storage there was no significant difference between the Cd loads in oysters from Groups S-WJ and HPP.

Effects of HPP. The low initial Cd levels observed in the HPP-treated oysters might be explained by certain changes that take place in oyster tissue when subjected to a high pressure system. Interestingly, HPP treatment has previously been reported to result in slight increases in the moisture content of oysters (He et al., 2002; Cruz-Romero et al., 2004). This is thought to be due to absorption of the fluid surrounding the oyster tissue inside the shell. Therefore, the lower Cd concentrations observed in the HPP-treated oysters compared to the SD and S-WJ oysters may have been partly a result of HPP-induced increases in moisture content. However, if water uptake did occur during HPP treatment, it did not significantly alter the initial weight of the oyster tissue, as illustrated in Table 2. Denaturation of proteins and decreases in protein content have also been reported to occur during HPP treatment (Cruz-Romero et al., 2004). Changes in the properties of Cd-bound proteins such as metallothioneins may have resulted in a release of Cd from the tissue. This could have contributed to the observed decreases in the Cd concentrations and total Cd loads of HPP-treated oysters compared to oysters in Groups SD and S-WJ. In addition to altering moisture and protein, HPP treatment results in elevated pH values and pH stability during storage compared to non-pressurized controls (Lopez-Caballero et al., 2000; He et al., 2002; Cruz-Romero et al., 2004). The increased pH is thought to be a result of the uptake of the seawater fluid surrounding the oyster tissue inside the shell; the pH of seawater is ~8.2 and the pH of untreated oyster tissue has been reported to be 6.45 (Cruz-Romero et al., 2004). Because pH has been reported to influence the form of Cd present and the binding activity of Cd, HPP-induced changes in pH may have affected the activity of Cd in oyster tissue (Luoma, 1983; Boyanov et al., 2003). Future work in this area should include an evaluation of the Cd, pH, protein, and moisture content in untreated samples, immediately after HPP treatment, and following washing, jar-packing, and storage.

5.7.4 Species

Various shellfish species were tested for tissue Cd concentrations and tissue weights in April 2006 at five Washington locations. Site locations included Eld Inlet (CHE), central Hood Canal (HAM), Willapa Bay (WILB), Totten Inlet (TOT) and Samish Bay (SAM). Results indicated that Cd concentrations in clams and mussels were well below 1 µg/g. Mean concentrations (µg/g) for geoducks, butter clams, manila clams and mussels (all sites combined) were 0.410 ± 0.056, 0.584 ± 0.100, 0.407 ± 0.015, and

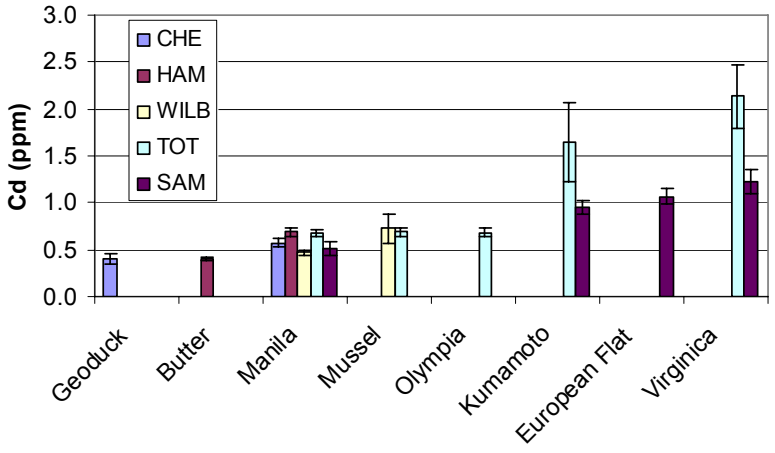


Figure 30. Cd concentrations in clams (geoduck, manila, butter), mussels, and oysters (Olympia, Kumamoto, Virginica, European Flat) from five Washington sites.

0.708 ± 0.106 respectively. Olympia oysters were also below 1 µg/g at 0.680 ± 0.044. Mean Cd concentrations (µg/g) in oyster composites harvested from only Samish Bay were 0.950 ± 0.075, 1.076 ± 0.083 and 1.220 ± 0.125 for Kumamotos, European Flats and Virginicas (Eastern) respectively (Figure 30). Species weights per individual are indicated in Figure 31.

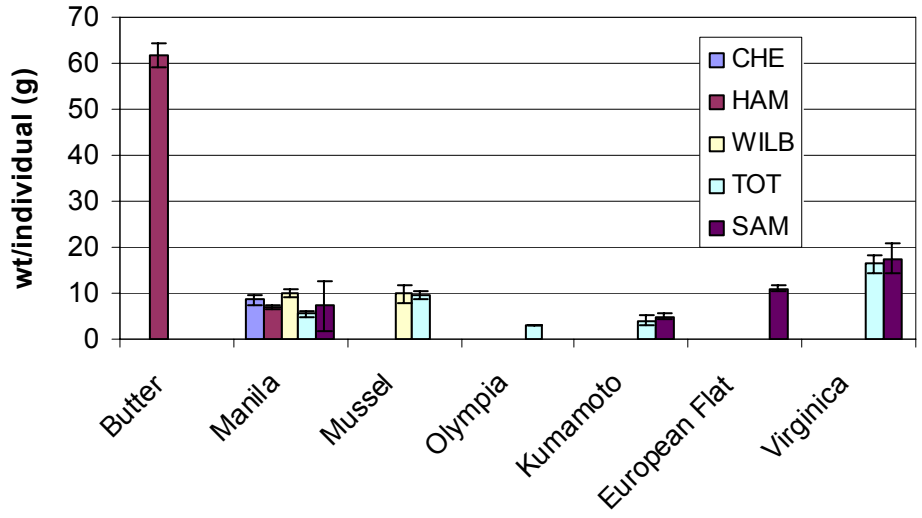


Figure 31. Weights per shucked individual for various shellfish species at five Washington locations.

Results were consistent with prior testing conducted by Taylor Shellfish Farms in September 2003 (Figure 32). For that study, shellfish composites were harvested from south Puget Sound with the exception of manila clams which were harvested from Hood Canal. Baudrimont, et al (2005) also found in French estuaries Cd bioaccumulation of oysters to be largely above the human consumption safety level, whereas it was lower in cockles and clams. They obtained similar results for Zn and Cu and suggested it was due physiological differences between the species and/or differences in the exposure of the organisms to variations in metal distribution between dissolved and particulate phases.

5.7.5 Oyster Liqueur

Results from OSUSRL research on the impact of various processing techniques on tissue concentration (Rasmussen and Morrissey, 2007) suggest that draining the oyster nectar prior to testing increases cadmium concentrations by an average of 54.5%. Oysters tested for this study included the nectar as per instructed in Sections 6.1.1.6 Sample Type and 7.2.4.4 Removal of Edible Tissue in the *National EPA Guidance for Assessing Chemical Contaminant Data for*

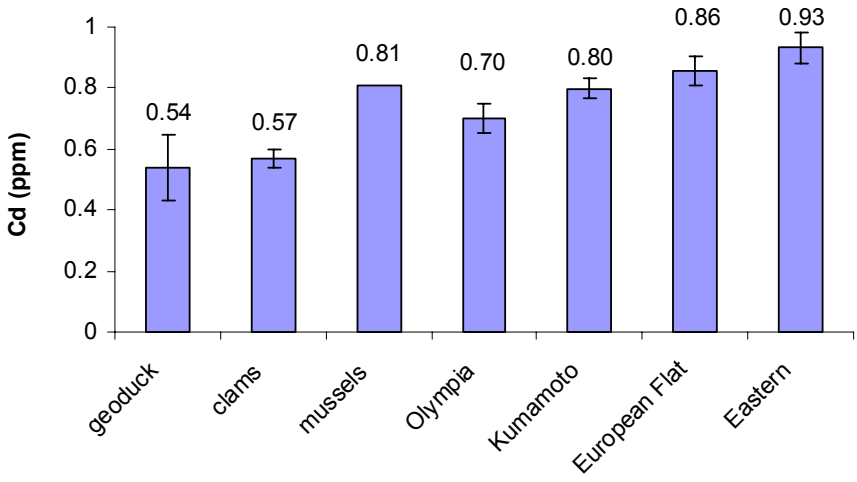


Figure 32. Cd concentrations in Puget Sound shellfish species. Data source: Taylor Shellfish Farms.

Use in Fish Advisories in addition to other sources and recommendations obtained prior to initial sample collection in September 2004.

During the summer of 2007, PSI and Integral Consulting contacted Washington State Department of Health (WDOH, 2007) to request their assistance in obtaining information from Hong Kong's Food and Environmental Hygiene

Department (FEHD) regarding laboratory procedures used to test cadmium levels in shellfish. In July, 2007, Hong Kong's FEHD sent information to WDOH indicating that they drain the oyster nectar prior to testing for cadmium in shellfish. In order to determine how the difference in laboratory testing procedures impacts Cd concentration and ultimately, shellfish grower's ability to export product to Hong Kong in light of their 2-µg/g import restriction, additional shellfish sampling was conducted.

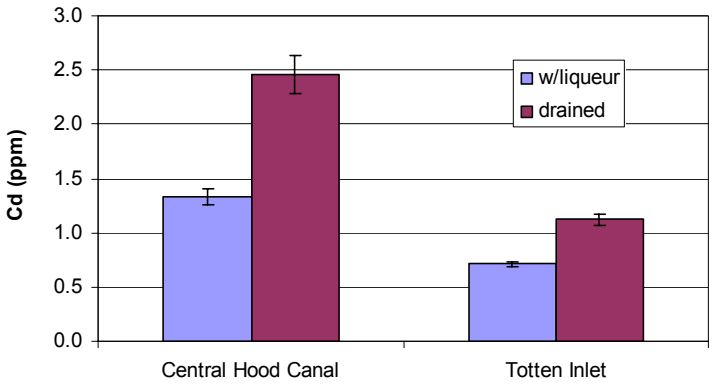


Figure 33. Average tissue Cd concentrations in oysters tested with and without the oyster liqueur.

In December, 2007, oysters were collected from south Puget Sound (Totten Inlet) and central Hood Canal to further assess the effect of including or draining oyster nectar (liqueur) in homogenized samples prior to analyzing tissue cadmium concentrations. Similar to OSU's results, Cd concentrations were found to be significantly higher in oysters whose liqueur was drained prior to homogenization than oysters whose liqueur was included in the homogenate ($p=0.04$, t-test assuming equal variances). In oysters from central Hood Canal, average tissue Cd concentrations were 1.33±0.07 µg/g with the liqueur included and 2.46±0.18 after the liqueur was drained (85.2% increase). In Totten Inlet, average tissue concentrations in oysters were 0.71±0.03 µg/g with the liqueur and 1.12±0.05 µg/g without (57.0% increase) (Figure 33).

Mean weights per shucked oyster (liqueur included) were 34.25±3.74 g at central Hood Canal and 51.95±5.22 g at Totten Inlet. The amount of liqueur per oyster was 17.23±2.27 g at Hood Canal and 22.97±3.20 g at Totten Inlet. The mean Cd concentration in the drained oyster liqueur was 0.063±.0005 µg/g for Hood Canal oysters and 0.029±.0199 µg/g for Totten Inlet oysters. The percent nectar content of Hood Canal oysters was 47.096±3.32 % and 42.546±2.77 % for those harvested from Totten Inlet.

If a 57.0% increase in tissue Cd concentration is applied to the 117 oyster composites tested in September 2004, 40.2% of the composites (47/117) would exceed Hong Kong's 2 µg/g ML. This percentage is much higher than 17.1% (20/117), or the percentage of composites that exceeded Hong Kong's import restriction when the liqueur was included in the homogenate prior to testing. The 57.0% percent increase in Cd concentration, however, applies to oysters harvested from Totten Inlet and may be too conservative for oysters located in other regions, particularly central Hood Canal that experienced an 85.2% increase in Cd after the liqueur was drained. In addition, the increase in Cd concentration likely varies not only by geographic region, but also by season as the percentage of oyster tissue and liqueur fluctuate.

6.0 DISCUSSION

One of the goals of this project was to define geographic locations, culture methods, shellfish species, harvest times, and additional factors that affect cadmium concentrations in an effort to minimize cadmium residues in shellfish products. To help achieve this goal a conceptual site model (CSM) was developed to identify potential cadmium sources, determine transport pathways, and attempt to explain why certain growing areas have higher cadmium tissue concentrations than others.

Generating a CSM for every sampling area in the study is beyond the scope of this project. Therefore, a CSM was developed focusing on Hood Canal since it is an area where the majority of oyster tissues exceeded the maximum cadmium limits established by many international regulatory agencies. On average, 58% of all US Pacific coast sampling stations in this study exceeded a 1-ppm cadmium ML in oyster tissue. However, only 15% exceeded the 2-ppm cadmium ML (Table 4-1). Of these, 60% (6 out of 10 stations) were from the Hood Canal area in Washington State. Potential health risks to vulnerable populations consuming oysters that exceed the 2-ppm cadmium ML are discussed in detail in Appendix B. A significant number of oyster growers in the Hood Canal area export their product to international markets, and these exceedances pose an economic barrier to international commerce (see Appendix C for a more in-depth analysis).

Most of the physical data for the Hood Canal CSM are from the Hood Canal Dissolved Oxygen Program (HCDOP). The HCDOP Integrated Assessment and Modeling Study (IAMS) was a three-year study (2004 to 2007) that analyzed marine, freshwater, and biota monitoring data and used a computer model to quantify the role the various natural processes and human actions play in varying the concentrations of dissolved oxygen in Hood Canal.

6.1 HOOD CANAL SITE DESCRIPTION

Hood Canal, a glacially carved, hook-shaped fjord, extends 60 miles southwards from Puget Sound's Admiralty Inlet (Hull and Bryan 2005). From the mouth to the head, canal bathymetry varies from shallow tide flats to more that 600 feet, and its width changes from one-half to four miles wide. A 150-foot-deep sill is located at the north end at the entrance of the canal and constricts the flow in and out of the Canal. Five major rivers from the eastern slopes of the Olympic Mountains and the western slopes of the Kitsap Peninsula drain into the canal. At the southern end there is a narrow watershed that drains several small streams directly into the hook area. Communities such as Belfair, at the head of the Canal, and Hoodsport, on its western shore, are the only major urban developments. The only large scale industrial development is the Bangor Naval Submarine Base located on the northeastern shore of the Canal. Most of the shoreline development is comprised primarily of small, narrow residential parcels along the northerly and southerly shorelines east of the Great Bend and near the head of the canal. Currently, there are an estimated 54,000 residents in the watershed with an estimated seasonal increase of several thousand persons during summer. All sewage is processed using onsite or clustered treatment systems with in-soil effluent dissipation.

6.2 SOURCES OF CADMIUM IN HOOD CANAL

The historic literature suggests background levels of cadmium in uncontaminated, nonbiological compartments extend over several orders of magnitude. Concentrations (ppb) of cadmium ranged from 0.05 to 0.2 in freshwater, up to 0.05 in coastal seawater, from 0.01 to 0.1 in open ocean seawater, up to 5,000 in riverine and lake sediments, 30 to 1,000 in marine sediments, 10 to 1,000 in soils of nonvolcanic origin, up to 4,500 in soils of volcanic origin, 1 to 600 in igneous rock, up to 100,000 in phosphatic rock, and 0.001 to 0.005 $\mu g/m^3$ in air (Korte 1983). Background levels of cadmium in crops and other plants are usually <1.0 mg/kg (ppm).

It is unlikely that human activity currently contributes to the elevated cadmium levels in Hood Canal. Potential atmospheric and industrial sources are either absent or highly managed (as in the case of the Naval Base). Population densities are light to modest, there is no mineral extraction, and agricultural practices (mainly timber harvest) are confined to limited areas of the watersheds. However, a number of natural sources and historic human activity could account for the elevated cadmium levels in oysters from Hood Canal (as well as other regions on the U.S. and Canadian west coast).

- **Upland sources (rivers and groundwater) --** As was noted above, weathering of cadmium minerals contained in rocks is a significant source of these releases to water in rivers and the ocean. However, there are no reports of elevated cadmium levels in surface and ground waters entering Hood Canal. Elsewhere, runoff associated with past mining activities (such as placer mining) could be contributing increased loads of cadmium and other metals to marine water bodies.

- **Upwelling of bottom waters rich in cadmium --** There is a clear relationship between upwelling-favorable wind forcing and very surface water Cd concentrations. Also, observations off the Oregon coast demonstrated the Cd content of nearshore water was very similar to that of upwelling offshore source waters, suggesting efficient exchange of waters between deeper water layers and the very nearshore region (Takesue and van Geen, 2002). Hood Canal is strongly influenced by upwelled water driven down the Strait of Juan de Fuca by on-shore currents. This is a probable source of cadmium enrichment in Canal waters.

- **Phytoplankton blooms --** In marine diatoms, the metals cadmium, cobalt and zinc can functionally substitute for one another to maintain optimal growth rates. This effect is at least partly due to metal replacement in the metal-binding site of the enzyme carbonic anhydrase. Because of the low concentrations of many essential trace metals in sea water, it is likely that Cd carbonic anhydrase (CDCA) and perhaps other metalloenzymes in marine organisms use unusual metals for activity and contribute to trace-metal concentration and cycling in the oceans. CDCA is also controlled in part by the availability of Cd and carbon dioxide in sea water (Lane, et al 2005). Therefore, in Hood Canal cadmium enriched phytoplankton may be contributing to either elevated particulate or dissolved Cd in surface waters.

- **Suspended sediments --** No relationship was observed between TSS and tissue Cd concentration (r_s=0.02, p=0.88) in Netarts Bay in Winter 2005. Cadmium concentrations in seston ranged from 0.000133 mg/kg at Netarts Bay in Winter 2005 to 0.66 mg/kg at Eld Inlet in Summer 2005 (Figure 18). Correlation analysis was not conducted for Cd in seston levels due to the large proportion of data that was undetectable, particularly in Fall 2005.

6.3 CADMIUM IN SHELLFISH

Under controlled laboratory conditions, measurements of kinetic uptake rates in seven populations of Pacific oyster seed indicate the main source of cadmium is from the dissolved phase. The relative contribution of cadmium from the dissolved phase to oyster tissues ranged from 4% to 92%. This is a very large range; however, dissolved cadmium contributions were higher than 73% on five out of seven oyster populations. Cadmium uptake from seston ranged from 8% to 96% where in most cases food contributed less than 27% cadmium. Additional information on laboratory studies of cadmium uptake and depuration is provided in Appendix A.

Field observations reviewed in this report found no statistically significant correlations between Cd concentrations in shellfish tissues and measurements of Cd in sediments, total carbon in sediments, total suspended solids, or total Cd in surface water. Factors identified that have a significant impact on Cd concentrations included:

- **Species or type of shellfish cultured** -- various molluscan shellfish types, including clams (manila, butter, native littleneck), mussels, oysters (Pacific, Olympia, Kumamoto, Virginica, and European flat), and geoduck were sampled at five Washington locations; of all oyster species, Olympia oysters had the lowest Cd body burdens.
- **The culture or growing methods employed** -- oyster seed grown at five Washington locations in off-bottom aqua-purses (AP) had significantly higher Cd concentrations than oysters gown in bags on-bottom (BOB)
- **Seasonality of harvest** – field observations indicated reduced Cd concentrations during summer months at most sampled stations with twofold to threefold changes in concentration occurring during the sampling period; similar variations were reported off the California coast in mussels (Ouellette, 1981).
- **Processing oysters for consumers** – tests of various processing techniques demonstrated that Cd concentrations can be reduced by 35% to 53% in oysters through high-pressure processing, washing, and jar-packing (Rasmussen and Morrissey, 2007).

7.0 HEALTH RISK

Potential health risks associated with consumption of Cd in Pacific oysters of the U.S. West Coast were evaluated using a probabilistic method. Two populations, a Native American tribe and an Asian and Pacific Islander group, included high-end oyster consumers relative to the third population, a typical

US. consumer. Assuming consumers are not exposed to significant Cd from other sources. Consumption of Cd in oysters from areas sampled in Washington is not likely to present a health hazard. However, taking into account Cd intake via cignrct1es and dietary sources other than oysters, Cd intake may approach or exceed the tolerable daily intake for some populations.

8.0 ECONOMIC RISK

A companion report on “Economic Effect to Hood Canal Producers of Reduction in Oyster Exports Due to Cadmium” is attached as Appendix C. This report presents: 1) an assessment of markets and revenues of Hood Canal oyster producers, and 2) and analysis of the revenue losses resulting from Hong Kong (and potential future international markets) restrictions on cadmium in oysters.

The Washington Office of Shellfish and Water Protection certifies 63 companies to harvest in the Hood Canal. These shellfish companies represent roughly 19 percent of the shellfish companies in Washington, and about 5 percent of annual Washington production of oysters. The oyster industry in the Hood Canal is characterized by the operation of numerous shellfish companies that produce a small amount of product relative to the Washington industry as a whole. More than half of the survey respondents produce oysters that are shipped to states outside Washington and two survey respondents produce oysters that are shipped to states external to the West Coast. The Asian ports of Hong Kong and Taipei continue to receive the bulk of exports; however, the prominence of other Asia ports is growing as it becomes easier to ship products directly to these markets. Nevertheless, four other producers surveyed plan to ship internationally in the future, probably in response to the strong international market in the past few years.

Hong Kong cadmium regulations have already resulted in revenue losses and additional costs to Hood Canal producers. One producer producer’s shipment was rejected by an international wholesaler based only on the fact that the oysters came from Hood Canal; and another company now conducts regular tests for cadmium. Other Asian countries have cadmium limits similar to Hong Kong’s, and in some cases even more stringent limits, though they are apparently not always well enforced. Hood Canal oyster producers exporting internationally face the risks associated with stronger enforcement of exiting regulations and the promulgation of new regulations because the

concentration of cadmium in oysters is naturally above the 2.0 ppm limit in most of the Hood Canal. The total potential losses to the Hood Canal oyster producers are estimated to be between $9,000 and $75,000 annually depending on the strength of the international and domestic markets if oysters are diverted to domestic markets. Revenue losses to Hood Canal are expected to be limited in the future for a number of reasons:

- Most Hood Canal operations are small and unlikely to ship product internationally.
- Only a small percentage of annual oyster production from Hood Canal goes to the international market.
- Many of the large operations harvest from sites outside the Hood Canal where cadmium Concentrations are less.

Mitigating actions available to producers that ship to the international market include the following:

- Ship to international countries with less stringent or non-existent cadmium regulations.
- Test for cadmium in oysters before shipping. A greater awareness of the cadmium threshold will prevent the one-time loss of shipments from the rejection of oysters from international ports.
- A Hood Canal company planning to enter the export market should test for cadmium. Findings of high levels of cadmium in the oysters suggests that in the short run, the company should focus on business relationships with wholesalers that ship to Taipei or Southeast Asia.
- Develop a method for reducing or minimizing cadmium residues in oysters prior to shipping. The cost to test oysters is not high compared to potential losses due to product rejection. However, the cost of frequent testing may be significant. Exploring methods to minimize cadmium residue in oysters is a long-run strategy that may address the problem in case the international market becomes particularly strong.

9.0 OUTREACH AND EXTENSION SERVICES

One of the primary means of providing outreach and extension services to the shellfish industry, high risk consumer groups, regulatory agencies and scientific community will be to distribute a copy of the Final Report to members within each of these groups. In addition to distributing project results electronically or as a hard copy, conference presentations and publications in scientific journals have also been used to further disseminate findings to the community. An ongoing list of individuals and organizations expressing interest in obtaining a copy of the Final Report is being maintained and continuously updated. To date, the list contains over 60 contacts. Report availability will also be announced in the Pacific Coast Shellfish Grower's Association (PCSGA) *Longlines* Newsletter and broadcasted on both the PCSGA and Pacific Shellfish Institute's web-sites.

Publications and conference presentations have been conducted to provide outreach and extension services to the shellfish industry, regulatory agencies, tribes and the scientific community. Peer-reviewed journal articles were published as well. Additional scientific journal publications are anticipated by project completion.

Publications completed in 2007 include:

> Rasmussen, R. S., Morrissey, M. T. and D. Cheney. 2007. Effect of Age and Tissue Weight on the Cadmium Concentration in Pacific Oysters (*Crassostrea gigas*). *Journal of Shellfish Research*, Vol. 26, No. 1, 173-179.
>
> Rasmussen, R. S. and M. T. Morrissey. 2007. The Effects of Processing Methods and Storage on Cadmium Levels in Pacific Oysters (*Crassostrea gigas*). *Journal of Aquatic Food Product Technology*.

Conference presentations delivered in 2007 include:

Rasmussen, R. S. The Effects of Processing Methods on Cadmium Levels in Pacific Oysters (Crassostrea gigas). 58th Annual Meeting of the Pacific Fisheries Technologists Conference. Hermosillo, Sonora, Mexico. February, 2007.

Christy, A. E. A Survey of Cadmium in Pacific Oysters: Spatial Distribution, Influencing Factors and Ways to Minimize Concentrations. Georgia Basin Puget Sound Research Conference. Vancouver, British Columbia. March, 2007.

Stupakoff, I. A Survey of Cadmium in Pacific Oysters (Crassostrea gigas) of the United States West Coast: Accumulation Pathways, Subcellular Distribution and Implications for the Shellfish Industry. Coastal Zone 2007. Portland, Oregon. July, 2007.

Christy, A. E. Characterization of the Cadmium Health Risk, Concentrations and Ways to Minimize Cadmium Residues in Shellfish. Pacific Coast Shellfish Growers Association/National Shellfisheries Association (PCSGA/NSA) 58th Annual Conference. Tacoma, Washington. October, 2004.

Christy, A. E. A Survey of Cadmium in Washington Pacific Oysters (*Crassostrea gigas*) and its Implications to the Shellfish Industry and Human Health. Presented to Washington Department of Health at the Annual Board Members Meeting. Olympia, Washington. July, 2005.

10.0 REFERENCES

ATSDR. 1999. Toxicological profile for cadmium. U.S. Public Health Service, Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Baudrimont, M., J. Schafer, V. Marie, et al. 2005. Geochemical survey and metal bioaccumulation of three bivalve species (*Crassostrea gigas, Cerastoderma edule* and *Ruditapes philippinarum*) in the Nord Medoc salt marshes (Gironde estuary, France). Sci.Total Environ. 337(1-3):265-280.

Christy, A. E. 2005. A Survey of Cadmium in Washington Pacific Oysters (*Crassostrea gigas*) and its Implications to the Shellfish Industry and Human Health. M. S. Thesis, The Evergreen State College.

Codex, 2006. CODEX ALIMENTARIUS COMMISSION report, Twenty-ninth Session, International Conference Centre, Geneva, Switzerland. JOINT FAO/WHO FOOD STANDARDS PROGRAMME.

Elinder, C. G., and Jarup, L. 1996. Exposure and Health Risks: Recent Findings. *Ambio*. 25:5, 370-373.

HKFEHD 2007. Regulation 3. Prohibition of sale etc. of food containing metals except where naturally present up to certain limits. Hong Kong Food and Environmental Hygiene Department. Food Adulteration (Metallic Contamination) Regulations. Accessed May 29, 2007. http://www.fehd.gov.hk/safefood/foodlaw_mc.html.

Hull T.E. and A. Bryan 2005. Solving Hood Canal's Low Oxygen Problem, The Onsite System Factor. Accessed June 14, 2007. http://www.psat.wa.gov/Publications/hood_canal/hood_canal_onsite_factor_2.pdf

Jin, Y. H., Clark, A. B., Slebos, R. J. C., Al-Refai, H., Taylor, J. A., Kunkel, T. A. et al. 2003. Cadmium is a mutagen that acts by inhibiting mismatch repair. *Nature Genetics*. 34, 326-329.

Johnson, M. D., Kenney, N., Stoica, A., et al. 2003. Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. *Nature Medicine*. 9:8, 1081-1084.

Korte, F. 1983. Ecotoxicology of cadmium: general review. Ecotoxicol. Environ. Safety 7:3-8.

Kuhnlein, H. V. and Chan, H. M. 2000. Environment and Contaminants in Traditional Food Systems of Northern Indigenous Peoples. *Annu. Rev. Nutr*. 20, 595-626.

Lane, T.W., M.A. Saito, G.N. George, I.J. Pickering, R.C. Prince, and F.M. M.Morel. 2005. A cadmium enzyme from a marine diatom. *Nature*, 435:p. 42.

NOAA (National Oceanic and Atmospheric Administration) 1998. Cadmium Levels in Bivalve Molluscs from the U.S. National Oceanic Atmospheric Administration Mussel Watch Project, 1986-1998.

NOAA (National Oceanic and Atmospheric Administration) 1999. Sediment Quality Guidelines Developed for the National Status and Trends (NS&T) Program.

NTP. 2005. Report on carcinogens, eleventh edition. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Washington, DC.

Patrick, G. 1996. Puget Sound Ambient Monitoring Program: 1992 and 1993 Shellfish Chemical Contaminant Data Report. Washington State Department of Health, Office of Toxic Substances, Olympia, WA.

PSEP 1997. Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound. Prepared for the U.S. Environmental Protection Agency Region 10, Office of Puget Sound, Seattle, WA and Puget Sound Water Quality Action Team, Olympia, WA. King County Environmental Laboratory, Seattle, WA.

PSI. 2004. Sampling Plan for Objective #1: Comprehensive Sampling of Cadmium Concentrations in Pacific West Coast Oysters. Prepared by the Pacific Shellfish Institute for the Washington State Department of Health, Office of Environmental Health Assessment, Olympia Washington, August 26, 2004 and revised September 3, 2004.

Pyron. 2006a. Data Review Report (AmTest). Characterization of the Cadmium Health Risk, Concentrations and Ways to Minimize Cadmium Residues in Shellfish. Prepared for Integral Consulting Inc by Pyron Environmental, Inc. 3530 32nd Way NW, Olympia, WA 98502. (August 18).

Pyron. 2006b. Data Review Report (Brooks Rand). Characterization of the Cadmium Health Risk, Concentrations and Ways to Minimize Cadmium Residues in Shellfish. Prepared for Integral Consulting Inc by Pyron Environmental, Inc. 3530 32nd Way NW, Olympia, WA 98502. (August 23).

Ouellette, T. R. 1981. Seasonal Variation of Trace-Metals in the Mussel *Mytilus californianus*. Environmental Conservation, 8(1):53-58.

Rainer, J. S. & R. Mann. 1992. A comparison of methods for calculating condition index in eastern oysters *Crassostrea virginica* (Gmelin, 1791). J. Shellfish Res. 11(1):55-58.

Rasmussen, R. S. and M. T. Morrissey. 2007. The Effects of Processing Methods and Storage on Cadmium Levels in Pacific Oysters (*Crassostrea gigas*). Journal of Aquatic Food Product Technology.

Rasmussen, R. S., Morrissey, M. T. and D. Cheney. 2007. Effect of Age and Tissue Weight on the Cadmium Concentration in Pacific Oysters (*Crassostrea gigas*). Journal of Shellfish Research, Vol. 26, No. 1, 173-179.

Satarug, S., Haswell-Elkins, M. R., and Moore, M. R. 2000. Safe levels of cadmium intake to prevent renal toxicity in human subjects. *Br. J. Nutrition*. 84, 791-802.

Satarug, S., Ujjin, P., Vanavanitkun, Y., Baker, F. R. and Moore, M. R. 2004. Influence of body iron store status and cigarette smoking on cadmium body burden of healthy Thai women and men. *Toxicology Letters*. 148, 177-185.

Schallie, K. 2001. Results of the 2000 survey of cadmium in B.C. oysters. Presented at: Cadmium and Oysters Workshop. March 6-7, Institute of Ocean Sciences, Sidney, BC.

Sullivan, M. F., Hardy, J. T., Miller, B. M., Buschbom, R. L., and Siewicki, T. C. 1984. Absorption and Distribution of Cadmium in mice Fed Diets Containing either Inorganic or Oyster-Incorporated Cadmium. *Toxicology and Applied Pharmacology*. 72, 210-217.

Suquamish Tribe 2000. Suquamish Tribe. 2002. Fish Consumption Survey of the Suquamish Indian Tribe of the Port Madison Indian Reservation, Puget Sound Region, The Suquamish Tribe, Suquamish, WA.

Takesue, R.K. and van Geen, A. 2002. Nearshore Circulation during Upwelling Inferred from the Distribution of Dissolved Cadmium off the Oregon Coast. Limnology and Oceanography, 47(1):176-185.

Toy, K.A., Polissar, N. L., Liao, S. and Gawne-Mittelstaedt, G. D. 1996. A Fish Consumption Survey of the Tulalip and Squaxin Island Tribes of the Puget Sound Region, Tulalip Tribes, Department of Environment, Marysville, WA.

USEPA (United States Environmental Protection Agency) 1980. Food and Nutrient Intakes of Individuals in One Day in the United States: Spring 1977, National Food Consumption Survey 1977-1978, Preliminary Report No. 2, Washington , D.C.

USEPA (United States Environmental Protection Agency) 2004. List of Drinking Water Contaminants and MCLs and Current National Recommended Water Quality Criteria. United States Environmental Protection Agency, Office of Water.

USFDA (United States Food and Drug Administration). 1993. *Guidance Document for Cadmium in Shellfish*. U.S. Department of Health and Human Services, Public Health Service, Office of Seafood (HFS-416), 200 C Street, SW, Washington, D.C. 20204. 44 pages.

USFDA (United States Food and Drug Administration). 2005. .04 Action Levels, Tolerances And Guidance levels for Poisonous or Deleterious Substances in Seafood. Chapter II Growing Areas. IV. Guidance Documents. National Shellfish Sanitation Program. Center for Food and Applied Nutrition. http://www.cfsan.fda.gov/~ear/nss3-42d.html. Accessed August 28, 2008.

Vahter, M., Berglund, M., Aksesson, A., and Liden C. 2002. Metals and Women's Health. *Environmental Research*. Section A. 88, 145-155.

WDOE (Washington Department of Ecology) 2005. Marine Water Quality Monitoring – Searchable Database. Washington Department of Ecology, Olympia, WA. http://www.ecy.wa.gov/programs/eap/mar_wat/mwm_intr.html

WDOH (Washington Department of Health) 2003a. Cadmium and Lead Results from Hood Canal, Washington. Report prepared by AmTest Laboratories for the Department of Health – Food Safety and Shellfish Programs. July, 2003.

WDOH (Washington Department of Health) 2003b. Cadmium Sampling Results from South Puget Sound, Washington. Report prepared by AmTest Laboratories for the Department of Health – Food Safety and Shellfish Programs. September, 2003.

WDOH (Washington Department of Health). 2007. Oyster-related illnesses last year prompt summer health advisory for recreational harvesters. May 15, 2007 (News Release 07-072).

WHO/FAO (World Health Organization/Food and Agriculture Organization) 1989. Evaluation of certain food additives and contaminants. Thirty third report of the Joint FAO/WHO Expert Committee on Food Additives, Technical Report Series 776, World Health Organization, Geneva, Switzerland.

11.0 APPENDICES