BIOACCUMULATION AND PUBLIC HEALTH IMPLICATIONS IN TISSUE OF DUNGENESS CRAB (*METACARCINUS MAGISTER)* CAUGHT WITHIN THE WASHINGTON COASTLINE AND HOOD CANAL.

by

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Abstract

Bioaccumulation and public health implications in tissue of Dungeness crab (*Metacarcinus magister)* caught within the Washington coastline and Hood Canal.

Kody Ludwig

Dungeness crab harvest supports key economic, recreational, and cultural significance for tribal and non-tribal populations in Washington state. Anthropogenic activities such as industrial and municipal discharges, boating and vehicle related phenomena, as well as stormwater runoff, have potentially contributed to heavy metal contamination, especially arsenic (As) accumulation, in these marine ecosystems. Heavy metals are harmful pollutants to humans and environmental health. The purpose of this study is to evaluate the inorganic arsenic (iAs) accumulation levels in Dungeness crab (*Metacarcinus magister)* edible tissues caught along the Washington outer coast and Hood Canal, as well as evaluate if consuming these crabs represent a potential human health risk. Contamination levels were compared between sampling sites, and between tissue types (muscle and hepatopancreas). Evaluation of the significance of contaminant levels on human health were compared against EPA health screening levels. Twenty crabs were collected from the Washington outer coast, outside of the Quinault Indian reservation, and 15 crabs were collected within the Hood Canal, connected to the Puget Sound. In the Washington coastal crabs, estimated iAs ranges were as follows: (iAs: 15.52-71.12 µg/kg wet weight). In Hood Canal, iAs ranges were as follows (iAs: 7.17-91.88 µg/kg wet weight). These concentrations were determined to be below EPA human health screening standards, thus not contributing to a human health risk. Furthermore, iAs concentrations were comparable to concentrations found in previous Puget Sound reference areas with presumably low levels of contamination. There was not a significant difference in iAs concentrations in the muscle tissue between the Washington Coastal and Hood Canal caught crabs; however, the hepatopancreas was significantly lower in Hood Canal (m=22.37, SE=2.86 µg/kg wet weight) than the Washington coast (m=31.39, SE=2.47 µg/kg wet weight) therefore producing unanswered questions about possible urban influences. Moreover, iAs concentrations in the hepatopancreas were not significantly greater than their corresponding muscle tissue in both sampling locations. Correlation analysis also revealed a strong positive relationship (r =0.7766) in iAs concentrations between the muscle and hepatopancreas tissue from the Washington coast, while the muscle tissue and hepatopancreas from the Hood Canal revealed a moderate positive relationship (r= 0.4313) in iAs concentrations. This research demonstrates that overall, Dungeness crab consumption appears to have minimal human health risk in terms of inorganic arsenic concentrations in the Washington outer coast and in the Northern Hood Canal region.

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Introduction

Elevated levels of heavy metals in the environment are a worldwide concern because of their toxicity, high persistence, non-biodegradability, and tendency to accumulate in organisms (Cogun et al. 2017). Increasing trends in human populations and coastal development contribute to the increase in anthropogenic pollution load, which has become a major threat to marine habitats (Alam et al. 2012). Heavy metals can be accumulated by aquatic organisms through food, water, and sediment in the environment that can be transferred through trophic levels, become lethal, and ultimately lead to death (Lorenzon et al. 2001). In addition, heavy metals may be transferred to humans through seafood consumption (Lie at al. 2019). As a result, there has been a growing interest in determining heavy metal levels in the marine environment and attention has been drawn to the measurement of contamination levels in food supplies (Tariq et al. 1993).

Arsenic (As) is among the heavy metals that are found in the marine environment and are highly toxic even at low exposure rates to marine organisms and humans (Flora et al. 2012; Kapaj et al. 2006). Arsenic can enter the marine environment from a variety pathway including non-point sources such as surface water runoff, groundwater releases, and air deposition, as well as focal non-point sources, such as marinas and ferry terminals (Lanksbury et al. 2014). In addition, vehicle related phenomena such as road deposited sediments, resulting from the deterioration of concrete roads, can expose humans and animals to As, resulting in certain risks. For example, previous literature has found a high association between non-carcinogenic health risks with the presence of As in highly trafficked areas and living near industrial spaces (Liu et al. 2014). Arsenic is also introduced into the environment through anthropogenic industrial practices such as mining, wood processing, pesticide and herbicide application, waste disposal,

and smelting, along with left-over smelter slag being used for road ballast (Gawel et al., 2014; Smedley et al., 2002).Other forms of arsenic exposure to humans includes inhalation of industrial dusts, in drinking water, and in food, particularly seafood (Mudhoo et al. 2011). However, arsenic in seafood is typically present in its organic form as arsenobetaine (AsB), which is considered nontoxic to humans (Ballin et al.1994), while inorganic arsenic (iAs) is toxic to humans, but only represents between 0.5 and 1% of total arsenic in edible portions of most fish and shellfish (Francesconi et al. 1993). In general, arsenic has both carcinogenic and non-carcinogenic effects on humans, (Khan et al. 2018) and can affect almost all cellular processes and organ functions in the human body (Mohammad Abdul et al. 2015).

Given the various pathways by which heavy metals accumulate in marine environments, it's of high importance to measure the variability of their concentrations in urban and remote settings. Furthermore, in the case of seafood (shellfish), for many tribes throughout the Pacific Northwest, including the Quinault Indian Nation (QIN), these organisms represent more than a commercial product but are vital subsistence food source, a part of their cultural identity, and represent an overall heritage to their traditional lifestyle (Donatuto 2003; Crosman et al. 2019). Among the various marine ecosystems of the Pacific Northwest lies the Puget Sound estuary, which is the second largest in the nation, central to the region's identity, and part of an extensive watershed that traverses some of the region's most populated areas (Wallace et al. 2018). The Puget Sound provides key economic, recreational, and cultural resources for the region, most notably, seafood. Located to the west of the main basin of the Puget Sound is Hood Canal. This sub-basin is less developed than the other Puget Sound areas, however, this region still experiences anthropogenic pressures such as, towns and villages, small marinas, a bordering highway, a navy submarine base, and stormwater run-off that could potentially result in elevated

heavy metal (HM) contamination (Long et al. 2010). Degradation of this environment is detrimental, drawing concern over anthropogenic activities such as wastewater and stormwater pollution coupled with large amounts of rainfall, resulting in overflow into the marine environment.

In contrast to the Puget Sound, the Washington coast is less densely populated and considered more rural. Along the central coastline lies the Quinault Indian reservation, located on the southern end of the Olympic Peninsula. To our knowledge, this area remains unexplored for arsenic contamination. Thus, although being impacted by fewer anthropogenic activities, there are still questions about the potential contamination of this marine ecosystem due to highly trafficked roads, stormwater run-off, and boating activities.

In general, within Washington state (WA), crab fishing is an important commercial and recreational fishing activity. Particularly, the Dungeness crab, a crustacean, supports one of biggest commercial fisheries for tribal and no-tribal coastal economies (Hart 2023; Donatuto 2003; Skoggard 2001). As a crustacean, the Dungeness crab have been identified as an excellent marine pollution bioindicator (Ololade et al. 2008). However, the extent to which it accumulates heavy metals has not been thoroughly explored between the Puget Sound and Washington Coast, and no examinations (to our knowledge) have been conducted on Washington's outer coast.

Previous studies have used Dungeness crabs as bioindicators in determining areas of heavy metal pollution and found variable concentrations of arsenic throughout urbanized marine waters in WA. For example, organic arsenic concentrations in Dungeness crabs were found to range from 3440 µg/kg wet weight in Commencement Bay to 20,500 µg/kg in Port Susan and Port Gardner (Carey et al.2014); however, arsenic concentrations were deemed unharmful to human health. In comparison, arsenic concentrations found within Padilla/Fidalgo Bay ranged

between 1.8 and 26 (ug/kg wet weight) and were considered a potential human health concern under different health parameters; conversely, the lack of identifying total arsenic vs. inorganic left uncertainties (Johnson et al. 2000). However, to this day, as previously mentioned, no studies have assessed contamination levels along the outer Washington coast. With the lack of data on the extent of contamination along the WA coast and the variable conditions found in previous accumulation patterns throughout the Puget sound, this further reiterates the necessity for characterizing the heavy metal levels in the environment and in the edible Dungeness crab's tissues, to limit chronic exposure to this pollutant, and we speculate to prevent the possible deterioration of tribe's cultural identity in the contamination of this traditional food source. Lastly, it brings us the unique opportunity in the comparison of the Puget Sound vs the Washington coast, two distinct marine environments with variable anthropogenic pressures.

In this present study, we will investigate the levels of inorganic arsenic accumulation in using Dungeness crabs as bioindicators to determine the geographic extent and magnitude of these heavy metals in the Puget Sound (northern end of Hood Canal) and on the Washington coast outside of the Quinault reservation. Specifically, this thesis will examine the following research questions: Are inorganic arsenic concentrations in Dungeness crabs collected along the Washington coastline outside of the Quinault Indian reservation and within the Puget Sound (northern end of Hood Canal) above healthy standard recommendations for human consumption? Is there a significant difference in the concentrations of inorganic arsenic in the Dungeness crabs collected along the Washington coastline outside of the Quinault Indian reservation and within the Puget Sound (northern end of Hood Canal)?

Literature Review

1) Introduction

Estuarine and coastal ecosystems provide multiple ecological, social, and economic services such as fishing, tourism, and shipping (Girard et al 2017). Pollution associated with the industrialization of coastal areas remains one of the main threats to marine ecosystems and the possible loss of these services. Metal pollutants are of particular concern because coastal areas are generally prone to accumulation, not only in the sediment but also in the overlying waters. This literature review will begin with a general description of heavy metals and their biological relevance in the marine environment. The next section will include an overview of the lead and arsenic properties, sources, and pathways into the marine environment. Following this section, will be a summary of the human risk exposure to heavy metals through seafood consumption, toxicological effects of arsenic (As) and lead (Pb) on human health, and regulation standards of seafood. The next section will summarize how As and Pb affect Dungeness crab and pertinent crab biological processes necessary to understand these impacts. Finally, there will be a review of the analytical methodology used to quantify heavy metals in the environment, such as the use of bioindicators, and general introduction to instrumentation necessary for analysis. This review of relevant literature will help guide the better understanding of the necessity of quantifying heavy metals in recreational and subsistence consumed crustaceans in their relevance to causing possible human health risks.

Our original intent was to analyze both arsenic and lead within Dungeness crabs; however, due to methodology issues, only arsenic was analyzed. Therefore, to reflect the original intent of this study, this literature review includes both references to arsenic and lead.

1.2) Heavy Metals

Metal and metalloids, also known as heavy metals, form one of the main element clusters in the periodic table (Sigg et al.2014). The term “heavy metals” is a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm^{3,} or roughly 5 times or more greater than water (Hutton et al. 1986). In environmental studies, relevant characteristics to define metals and metalloids would be non-degradability, ability to be bioaccumulated, potential to be a source of nutrients, and speciation- dependent toxicity (Rainbow 2002). Nowadays the term “heavy metal” has been used to describe the chemical properties of metallic elements and metalloids which are toxic to the environment and humans (Ali et al. 2019).

1.2.1) Non-Essential vs Essential Metals

Heavy metals are distinctly classified as essential and non-essential, in regards to their roles in the biological systems (Ali et al. 2019). Essential heavy metals are important for living organisms and may be required in the body in lower concentrations. Non-essential heavy metals have no known biological role in living organisms and can cause negative effects in organisms at lower concentrations (Rainbow 2002). Examples of essential heavy metals (HM) are magnesium (Mg), iron (Fe), copper (Cu), and Zinc (Zn), while the heavy metals cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg) are toxic and regarded as biologically nonessential. Essential HMs play important physiological and biochemical roles in organisms as they may be part of the biomolecules such as enzymes, carbohydrates, and nucleic acids, which catalyze biochemical reactions in the body (Ali et al. 2019; Appenroth et al.2010). Being in excess or deficient of an essential heavy metal can harmful and lead to adverse effects at a cell, organ, or body level (Gaulier et al. 2019). However, the lists of essential heavy metals may be different for varying

groups of organisms such as human, plants, and microorganisms. It means a heavy metal may be essential for a certain group of organisms, but non-essential to others. Thus, the interactions of heavy metals among different organisms' groups are very complex.

1.3) Heavy Metals in Marine Ecosystems

1.3.1) Metallic Sources and Pathways

Heavy metals can emanate from both natural and anthropogenic processes and end up in different environmental compartments (soil, water, air) (Saleh et al. 2018). Natural sources of heavy metals to the marine environment include the weathering of soils and rocks, and volcanic discharges (Ali et al.2019). Anthropogenic sources of heavy metals into the marine environment include industrial wastes, agricultural runoff, municipal solid wastes in coastal landfill, mining activities, smelting, dredging activities, boating activities, geothermal discharges, and pollution found in storm water runoff (Akter et al.2005; Acosta et al.2010). Further, heavy metals are released to the atmosphere during mining, smelting, and other industrial processes return to land through dry and wet deposition (Ali et al. 2019). Regarding agricultural activities, the application of chemical fertilizers such as inorganic phosphate, can potentially contribute to the global transport of heavy metals (Ali et al. 2019). Overall, the global trends in industrialization have led to an increase in the anthropogenic share of heavy metals in the marine environment.

Heavy metals discharged into the aquatic systems by natural or anthropogenic sources during transport are distributed between the aqueous phase and sediments. Trace metals may exist in the dissolved phase as metal ions, dissolved inorganic metal- ion pairs, organic forms (dissolved organic matter) and as colloidal forms in both porewater and the water column (Smedley et al.2002). However, only a small portion of free metal ions stay dissolved in water

(Hou et al. 2013). As such, more than 90 percent of heavy metal load in aquatic systems have been found to be related to suspended particles and sediments (Amin et al.2009).

1.3.2) Lead – Properties, Uses and Origin

Lead is naturally present in the Earth's crust although rarely found in pure form but in ores with other metals (Cariou et al.2017). Lead's relatively stable isotopes are ^{204}Pb, ^{205}Pb, ^{207}Pb, and ^{208}Pb, the latter three of which represent the end product of radioactive decay chains from uranium, actinium, and thorium (Botte et al.2022). Lead can mainly be found in the environment in the form of salts ($PbCO_3$, $Pb(NO_3)$, $PbSO_4$), hydroxylated ($Pb(OH)_2$) or ionized (Pb^{2+}) (Botte et al. 2022) through the deposition of particles. Lead is not an essential nutrient to any organism. It can either be organic, inorganic, or uniquely found in its metallic form in nature, but most frequently is present in its +2-oxidation state (O'Neil 1998; Botte et al. 2022).

A variety of geologic processes can cause natural lead enrichment, commonly in association with other metals, and disperse it into the environment. It's naturally introduced in marine environments by forest fires, volcanic activity, erosion, and transport processes (Cariou et al.2017). Weathering and erosion of Pb leads to dispersion at or near the Earth's surface. For example, chemical weathering concentrates lead to residual materials such as weathered ores and clay-rich soils, which may then be transported by running water and contribute to sediment loads in rivers, streams, and oceans (NAP 2017). Forest fires can mobilize Pb in topsoils into the atmosphere, as well as facilitate decreases in the porosity of the soil structure, thus facilitating accumulated ash runoff and wind erosion into nearby rivers, streams, and oceans (Baieta et al. 2022). Volcanic activity can result in particulate Pb, diverse in size and chemical composition, to be dispersed by prevailing winds and subsequently delivered to aquatic environments by wet and dry deposition (Sigel et al.2017). Atmospheric deposition and riverine transports ultimately

deliver Pb to the marine ecosystem, which serves as a storeroom for much of the Pb released from natural and anthropogenic sources (Sigel et al. 2017).

Anthropogenic sources introduce lead into the aquatic ecosystems from manufacturing processes, atmospheric deposition (combustion of leaded fuels; the burning of wood and coals), domestic wastewaters, and sewage (Nriagu et al. 1988). The key uses of lead are in lead- acid storage batteries in motor vehicles, lead alkyl compounds that are added to petrol to reduce knock in combustion engines, in pigments, lead paints, anti-fouling paints, and as stabilizers in plastics (Denton et al.1997;Wani et al.2015).

1.3.3) Lead Solubility in Water.

In the aquatic environments, Pb is primarily distributed in its dissolved forms, and the specific chemical conditions determines its speciation. In water, lead comes in the inorganic forms such as $Pb(OH)_2$, and $Pb(OH)_3$, and polymeric lead ions such as $Pb_2(OH)_3^+$ and $Pb_4(OH)_4^{4+}$(Hill 2005). Insoluble compounds include PbO, $PbCO_3$ and $PbSO_4$. However, most studies refer to the inorganic species of Pb simply as the Pb^{2+}ion. In seawater and high dissolved chloride systems, lead chloride complexes ($PbCl^+$, $PbCl_{3-}$ and $PbCl_2$ (aq)) are more prevalent than free Pb^{2+} when pH is below 7.5 (Powell et al. 2009). At alkaline pH and high chloride, dissolved lead is mostly present as aqueous lead-carbonate complexes, such as $PbCO_3$ and $PB(CO_3)_2^{2-}$ instead of PB^{2+}(Powell et al. 2009). However, lead is barely soluble in seawater and is readily absorbed by hydrous metal oxides, clay minerals and organic materials (Denton et al.1997). This is evident with only approximately 5 percent of lead in the aquatic systems being found in the dissolved form (O'Neil 1998). Lead residence time is estimated to be less than five years in the surface waters (Veron et al.1987), however in sediments and soils, its geochemical half-life is estimated about seven centuries (Semlali et al.2004).

1.3.4) Lead Accumulation in Sediment.

Due to lead's affinity for being absorbed by clay, organic matter, oxides and hydroxides of iron, manganese, calcium, and carbonates (Agah et al. 2009), it tends to accumulate in the sediments as these organic materials are deposited and sink to the bottom at the point of entry into the hydrosphere (Denton et al.1997). Sediments act as the main pool of metals in the aquatic environment (Ali et al.2019). They can act both as sources as well as sinks for heavy metals, releasing contaminated particles into the water column (Ali et al.2019). Thus, the sediments not only act as the carriers of contaminants, but also the potential secondary sources of contaminants in aquatic ecosystems (Calmano et al.1990). Changes in sediment chemistry, due to seabed disturbance, can result in contaminant remobilization. Subsequently, exposure to a different chemical environment could result in desorption and transformation of contaminants into more bioavailable or toxic chemical forms (Eggleton et al. 2004). Distribution of lead in sediments is affected by the chemical composition of sediments, grain size, and content of total organic matter (Azadi et al.2018). Therefore, the geochemical behavior of lead can vary depending on the type of aquatic ecosystem. Clay minerals for example can bind Pb^{2+}, particularly at acidic pH, but the bound lead can be displaced by cations, such as Ca^{2+}, when they are present at concentrations similar to that of Pb^{2+}(National Academies of Science 2017). Lead in the marine environment can also precipitate as lead sulphide, an insoluble compound, that often exists when bound to suspended sediment particulate matter (O'Neil 1998).

1.3.5) Arsenic - Properties of As, Uses, Origin of Source.

Arsenic (As) occurs naturally as a major constituent of more than 245 minerals, including elemental arsenides, sulfides, oxides, arsenates, and arsenites (Akter et al.2005). Although arsenic is often referred to as a metal, its classified chemically as a nonmetal or metalloid (Akter

et al.2005). Arsenic is mobilized naturally in the environment due to weathering of rocks, hydrothermal and geothermal activities, biological activities, and a range of anthropogenic activities. Arsenic can be mobilized by forest fires and volcanic activity through dry and wet deposition (Fowler et al. 2013). Arsenic can be found in the natural environment in both organic and inorganic forms, and several oxidation states (-3,0,+3, and +5) (Akter et al.2005). The main source of As in soils is the parent rock from which the soil was derived. Oxidation of arsenopyrite is the widespread mechanism for the distribution of arsenic into the environment (Flora et al. 2015). Arsenopyrite is formed under high temperatures in hydrothermal vents and a reduced anerobic environment, such as areas around buried plant matter or other nuclei of decomposing organic matter (Flora et al. 2015). Baseline concentrations in soil are generally found between 5-10mg/kg (Smedley et al. 2002).

Anthropogenic activities are the main sources of As in the environment, exceeding natural sources by a 3:1 ratio (Fowler et al. 2013). Anthropogenic sources of arsenic to the marine environment include agricultural runoff from use of fertilizers and pesticides, urban runoff, industrial waste, treated wood and timbers, manufacturing process, smelting, mining, and municipal solid waste (Denton et al.1997). About 90 percent of industrial As in the US is used for wood preservation, but As is also commercially used in the manufacture of paints, dyes, ceramics, glass, electronics, pigments, agricultural products (fungicides, herbicides, pesticides), animal feeds additives, and antifouling agents (Leonard et al 1991; Denton et al. 1997).

1.3.6) Arsenic Solubility in Water

Arsenic has a complex marine biogeochemistry that has important implications for its toxicity in the marine environments. Arsenic can occur in estuarine and marine waters in four valency states, +5, +3,0, and -3 (Moore et al. 2012). The dominate forms of inorganic arsenic is arsenite (

As III) or arsenate (As V) in relatively low concentrations (Smedley et al. 2002), with a concentration that is dependent on local geology, hydrology, and geochemical characteristics of the aquatic materials (Bhattacharya et al.1997). Arsenous acid (H_3AsO_3) is typically found in surface ocean waters (Cutter et al.2001). Two other organic forms of arsenic, methylarsonic acid (MMA) and dimethylarsinic acid (DMA) are also found in seawater (Smedley et al. 2002). That said, arsenic is typically depleted in surface waters of the open ocean. Total arsenic concentrations in open seawater usually show little variation and are typically 1.5 $\mu g/l^{-1}$(Neff et al. 1997). Total Arsenic concentrations in estuarine water are more variable as a result of varying river inputs, proximity to anthropogenic sources, and salinity or redox gradients, but are typically less than 4 $\mu g/l^{-1}$.

Once in the water, As can go through a complex series of transformations, including oxidation-reduction reactions, ligand exchange, and biotransformation (Smedley et al. 2002). Example of this include arsenic readily forming oxyanions in both oxidation states (As(V) ($HAsO_4^{2-}$ and H_2AsO_4-) and As III (H_3AsO_3)) under pH conditions between 6.5-8.5 found in ground water (Flora et al. 2015). At this pH range, most toxic heavy metal ions remain dissolved in solution. However, they may precipitate or co-precipitate as oxides, hydroxides, carbonates, or phosphates as the pH increases, unlike arsenic, which can form oxyanions but can still exist dissolved in solution at higher pH values. Thus, arsenic's relative mobility over a wide range of redox conditions makes it highly problematic in the environment (Smedley et al. 2002).

1.3.7) Arsenic Accumulation in Sediment.

In oceanic sediments, arsenic is usually concentrated in fine grain sediments, particularly those rich in organic matter, sulfide minerals, or phosphate, or iron oxides (Plant et al. 2003). Arsenic occurs in sediment mainly as arsenate, AsO_4^{3-} under oxygenated conditions (Flora et al. 2015). This form of arsenic is strongly absorbed onto clays, oxides/hydroxides of iron ($Fe_3(ASO_4)_2$) and manganese, and organic matter (Flora et al.2015; Andreae et al. 1989). In alkaline and calcareous sediment, the main form is $Ca_3(ASO_4)_2$ (Flora et al. 2015). Arsenite is the dominant dissolved species in reduced sediment layers (Neff et al. 1997). Bacteria in aerobic sediments can oxidize As (III) to As (V)(Masscheleyn et al.2002). Iron oxyhydroxides which are abundant in oxidized marine sediment, also can catalyze the oxidation of arsenite to arsenate (Masscheleyn et al. 1991). Absorbed or bound arsenic is less bioavailable than dissolved arsenic (Neff et al. 1997). Thus, much of the arsenic in oxidized marine sediments may not be bioavailable to sediment dwelling marine organisms (Neff et al. 1997). For example, arsenate can be reduced to arsenite in sediments, and if sulfur is abundant, most of the arsenic reacts to sulfide to form realgar (AsS) and inclusions in copper and zinc sulfides (Sadiq et al. 1990). These sulfides have low solubility and mobility (Sadiq et al. 1990).

Arsenic is most likely to be dissolved and mobilized from marine sediment at redox interfaces and during transitions in redox potential (Moore et al. 1988). During redox potential changes, arsenic is released into solution in the pore water due to the dissolution of iron and manganese oxides or sulfides, especially pyrite (Neff et al. 1997). The arsenic rich pore water may then be mixed into the overlying water column by sediment resuspension and transport or by bioturbation (Masschelleyn et al. 1991).

1.4) Human Exposure to Heavy Metals

Humans can be exposed to heavy metals via food, water, and to a much lesser degree via the air and through dermal contact (Akter et al. 2005). Lead and arsenic are of great concern primarily due to their high potential to accumulate in the food chain and cause harmful effects on organisms (Ikemoto et al. 2008). Through the consumption of edible marine organisms such as shellfish, heavy metals can be transferred through marine tissues to humans and cause adverse effects (Liu et al. 2019). Recently marine product consumptions have increased due to increased public awareness of their health effects and nutritional value (Stankovic et al. 2002). Fish and seafood are now recognized as important sources of protein for human health and provide omega-3 fatty acids, minerals, and vitamins (Stankovic et al. 2002). With the increasing consumption of shellfish in recent times occurring (Bentley 2019), so does the possibility of increased exposure of heavy metals in humans.

1.4.1) Trophic Transfer of Heavy Metals

Heavy metals being transferred to humans through the consumption of shellfish in a process known as trophic transfer (Liu et al. 2019). It refers to the phenomenon by which metal concentrations are transferred from one level in the food chain to the next (Hare et al. 2013). Heavy metals may be taken up by organisms such as plants, bacteria, fungi, phytoplankton that are situated at the base of the food chains. When such organisms are consumed by animals, a portion of the heavy metal is transferred into their gut membrane. As heavy metals are biomagnified efficiently, they have the potential to be present in high concentrations in top predators. Biomagnification is referred to as the rise in levels of pollutants along food chains, which leads to increased accumulation in successive trophic levels (Verma et al. 2023). This biomagnification process could consequently have adverse impacts on human health.

1.4.2) Human Health Implications of Lead Consumption

Lead (Pb) is considered toxic in most of its chemical forms that can affect humans whether it is inhaled through to air, ingested in water, or consumed in food sources (Assi et al. 2016). Exposure of lead induces clinic pathological changes through toxicity occurring in kidney and endocrine system (Patra et al. 2011). Due to its slow rate of elimination, harmful levels of lead can accumulate in tissues after prolonged exposure to low quantities. Exposure to lead is considered detrimental and associated with behavioral abnormalities, hearing deficits, neuromuscular weakness, and impaired cognitive functions (Flora et al.2012).

The amount of lead absorbed in humans via ingestion is affected by physical characteristics such as age, pregnancy status, fasting state, iron and calcium status, and the physio- chemical nature of the material being ingested, such as size of particles, solubility, mineralogy, and Pb species (WHO/FAO 2011). Transport of Pb to different body tissues (e.g., liver, kidneys, bone tissue) from the intestine is via red blood cells, where binding takes place between Pb and hemoglobin (Kumar et al. 2020). The half-life of Pb in blood and plasma is estimated to be 35- 40 days, whereas the Pb can reside in bone for up to 30 years, and concentrations of Pb in teeth and bone grow in proportion to age (Kabata- Pendias et al. 2015). Lead creates chemical bonds with thiol groups of proteins and Pb toxicity is believed to inhibit enzymes and subsequently interfere with homeostasis of Vitamin D, Magnesium, Calcium, and Zinc (Kumar et al.2020). Lead disrupts the maintenance of the cell membrane, and red blood cells with a damaged membrane become more fragile, thus resulting in anemia (White et al. 2007). Lead poisoning may also induce chronic liver damage and reduce protein synthesis (Yuan et al. 2014). Other organ and tissue systems affected due to lead toxicity are the nervous system, cardiovascular system, and reproductive systems (Yuan et al. 2014). Lead exposure can affect the renal system resulting in chronic renal disease and overall renal insufficiency (Yu et al.

2003). The reproductive system of both males and females is affected by Pb and can lead to changes in motility/morphology of sperm, infertility, miscarriages, and problems with development during childhood (Park et al. 2008). Lead affects the nervous system by interfering with the development of neuro chemicals, including neurotransmitters, organization of ion channels (Casarett et al.2008). Prenatal and childhood exposure are at higher risk of developmental disabilities, loss of cognitive abilities, behavior problems such as aggression, and psychiatric conditions like depression and anxiety (Park et al.2008; Brunton et al. 2007).

1.4.3) Human Health Implications of Arsenic Consumption

Non-essential metals such as arsenic, may cause a diverse range of toxic effects to humans at smaller doses, leading to acute or chronic toxicity. Along with general toxicities, potential carcinogenicity of metal compounds is a growing concern. In general, the toxicity of the metal is due to the chemical reactivity of the ions with cellular structural proteins, enzymes and membrane systems (Mahurpawar et al. 2015). The toxic effect of arsenic depends specially on its oxidation state and chemical species, among others (Oosthuizen et al. 2012). Depending on the type of arsenic exposure (acute or chronic) development of symptoms varies (Mohammed Abdul et al. 2015). Symptoms of acute exposure develop more quickly, whereas clinical symptoms of chronic exposure develop over prolonged periods of time (Mohammed Abdul et al 2015).

Toxicity of arsenic in its inorganic form have been known for decades with primary routes of exposure including drinking water (Mudhoo et al. 2011) and the ingestion of polluted foods. Chronic arsenic ingestion from drinking water has been found to cause carcinogenic and non-carcinogenic health effects in humans. Low to moderate levels of arsenic exposure (10-300 μg/L) through drinking water has adverse effects such as skin lesions, circulatory disorders, neurological complications, diabetes, respiratory complications, sarihepatic and renal

dysfunction including morality due to chronic disease (Chen et al. 2009). Ingestion of As may lead to tumors in various body parts such as skin, bladder, kidneys, lungs, and liver along with other circulatory and neurological complications (Mandal et al. 2001). Skin abnormalities such as skin lesions (melanosis, keratosis, and pigmentation) are key features of arsenic exposure (Rahman et al. 2009).

Neurological complications due to arsenic being distributed in the brain, can affect learning and concentration (Mundey et al.2013). Other common symptoms of neurological complications include paresthesia and pain/ numbness in the soles of feet, due to decreased capabilities of neurons to detoxify reactive oxygen species (Aoyama et al.2008). In severe conditions, inactivation of crucial enzymes that governs the cell function may be hindered. Moreover, the typical features of arsenic induced neurotoxicity can lead to gradual loss of brain function and activity over time (Mohammad Abdul et al. 2015).

Arsenic exposure affects the hematopoietic system including bone marrow, spleen and erythrocytes (blood cells) ((Mohammad Abdul et al. 2015) resulting in anemia, bone marrow depression, and the possibility of developing circulatory disorders (Szymanska- Chabowksa et al.2002). Arsenic can induce detrimental effects on the immune system inducing a range of autoimmune diseases including diabetes, atherosclerosis, and non-melanoma skin cancers (Banerjee et al. 2009). Arsenic is a well-known disruptor of the endocrine system, including thyroid, pancreas, and gonads (Mohammad Abdul et al. 2015), which can lead to the disruption of the secretion of key hormones such as cortisol, insulin, and growth hormone being secreted (Lu et al. 2011). Accumulation of arsenic in liver is prone to increase hepatic toxicity resulting in liver disease/failure and hepatic lesions (Kapaj et al. 2006). During the process of arsenic elimination through renal system (urine) accumulation of arsenic in kidneys can lead to chronic

kidney disfunction/diseases (Zheng et al. 2014). Arsenic can affect the reproductive and development of fetuses, as well as cause fertility issues in both genders (Mohammad Abdul et al. 2015). In addition, it's been recognized as a well known carcinogen since the late 17th century, and has shown the ability to induce tumors in humans' skin, lungs, bladder, liver, and prostates (Mohammad Abdul et al. 2015).

Overall, arsenic affects almost all cellular processes and organ functions in our body and can result in the development of cancer in various parts of the body.

1.4.4) Regulatory Standards For Seafood

Limits for heavy metal concentrations in food standards are set to minimize health impacts on consumers, by limiting the intake of contaminated foods. Regulatory authorities such as World Health Organization (WHO), the United States Food and Drug Administration (USFDA) and the Environmental Protection Agency (EPA) provide guidelines for dietary intake limits and maximum allowable values of specific contaminants in food. Data for seafood heavy metals collected in the current thesis were used to conduct risk assessments for consumption of the species measured. In shellfish, of the total arsenic concentrations In the edible parts, 85% to 90% of it is in the organic form, such as arsenobetaine (AsB), which is considered nontoxic to humans (Ballin et al.1994). The toxic species of most concern are inorganic arsenic, which has been found to consist of approximately between 0.5% -10% (Szteke 2015), and methylated organic arsenic forms such as monomethylarsonic acid (MMA) and dimethlyarsinic (DMA) (Johnson et al.2002). However, the inorganic arsenic content in shellfish may be highly variable (Szteke 2015). In addition, there currently are no state or EPA standards for MMA and DMA (Johnson et al.2002). In comparison, lead, however, is toxic regardless of inorganic or organic forms in shellfish tissues (Assi et al. 2016).

The EPA uses screening values (SVs), which are known concentrations of certain pollutants in fish or shellfish that are of potential public health concern, as threshold values in identifying levels of contamination in similar animal tissue collected from different environments to be compared to (EPA 2000). The exceedance of these SVs prompts an evaluation of human health risk. The following equation is used to calculate SVs for noncarcinogens:

$$SV_n = (RfD \times BW)/CR$$

SV_n = Screening value for a noncarcinogen (mg/Kg; ppm)

RfD = Oral reference dose (mg/kg/d)

BW = Mean body weight of general population or subpopulation (kg)

CR = Consumption Rates of organism for selected subpopulations (kg/d)

The reference dose (RfD) is an estimate of a daily oral exposure to the human population (including sensitive groups) that is likely to be without an appreciable risk of detrimental effects (cancerous or non-cancerous) during a lifetime (EPA 2012a). The RfD is expressed in mg/kg/day, whereas kg refers to the body weight of the individual ingesting the organism. EPA body weight (BW) values are the average body weights corresponding to various population groups (i.e., adult men and women, children, and adolescents) and are expressed in kg (See Table 1) (EPA 2000). Consumption rates (CR) are the mean daily consumption rates of the organism of interest by the general population or subpopulation of concern averaged over a 70-year lifetime and expressed in g/day. Table 2 shows a comparison of fish and shellfish consumption rates for various fisher populations within the general population and in several surveys of specific Native American tribal populations. These CR values are the results of the continued Survey of Food Intake by Individual (CSFII) survey conducted by the U.S.

Department of Agriculture, consisting of multistage surveys across the U.S. (USDA 1998) in addition to independent studies of surveying consumption rates for several different Native American tribes. The EPA currently recommends default fish consumption rates of 17.5 grams per day (g/d) for general and recreational fishers and 142.4 g/d for subsistence fishers. It should be noted that tribal population fish consumption studies show that Native American tribal members living near coastal and river-based communities can consume 3 to 22 times more fish than recreational fishers, as well as traditional native American subsistence fishing families may eat up to 30 times more fish and shellfish (Harris and Harper 1997). Therefore, the Native American subsistence fishing population should be treated as a separate group with a unique lifestyle, distinct from recreational and subsistence fishermen in the general U.S. population as well as from other Native American fisher populations (EPA 2000). The currently used EPA RfD for lead is 0.0004 mg/Kg (0.4 µg/kg) of body weight per day (EPA IRIS 2011). The RfD for Inorganic arsenic is 0.0003 mg/Kg (0.3 µg/kg) of body weight per day and based on hyperpigmentation, keratosis, and possible vascular complications in humans including sensitive groups. It should be noted, however, that the RfD methodology, by definition, yields a number of uncertainties perhaps by an order of magnitude (EPA 2012a). Overall, the EPA's recommended SVs were chosen as the health advisory benchmark for this study due to their giving full priority to the protection of public health, providing a direct link between fish consumption rate and risk levels, their general conservative estimates of increased risk, their incorporation of uncertainty factors that reflect various types of data sets through previous toxicology studies, and their inclusion of susceptible populations that could potentially be at greater risk than the general adult population due to frequently consuming greater quantities of locally caught fish and shellfish (Reinert et al. 1991).

Table 1.

Recommended Values for Mean Body Weight (BWs)

Variable	Recommended value	Subpopulation
BW	70 kg	All adults (U.S. EPA, 1999a)
	78 kg	Adult males (U.S. EPA, 1985b, 1990a)
	65 kg	Adult females (U.S. EPA, 1985b, 1990a)
	12 kg	Children <3 yr (U.S. EPA, 1985b, 1990a)
	17 kg	Children 3 to <6 yr (U.S. EPA, 1985b, 1990a)
	25 kg	Children 6 to <9 yr (U.S. EPA, 1985b, 1990a)
	36 kg	Children 9 to <12 yr (U.S. EPA, 1985b, 1990a)
	51 kg	Children 12 to <15 yr (U.S. EPA, 1985b, 1990a)
	61 kg	Children 15 to <18 yr (U.S. EPA, 1985b, 1990a)

Note. Body weight (BW) dose response variable used to calculate the Screen values for arsenic and lead suggested by EPA. Source: U.S. EPA 1995.

Table 2.

Fish Consumption Rates for Various Fisher Populations

Source	Recreational Fishers (g/d)	Subsistence Fishers (g/d)	Native American Subsistence Fishers (g/d)	Native Americans (g/d)	Basis for Consumption Rate
U.S. EPA	17.5 [a]	142.4 [a]	70 (mean) [b] 170 (95th percentile)[b]	NA	Fish consumption rate from 1994 and 1996 Continuing Survey of Food Intake by Individuals (CSFII)
Harris and Harper (1997)	NA	NA	540 (fresh, smoked and dried)	NA	Surveyed members of the Confederated Tribes of the Umatilla Indian Reservation
CRITFC (1994)	NA	NA	NA	59 (mean) 170 (95th percentile) 390 (99th percentile)	Surveyed members of the Umatilla, Nez Perce, Yakama, and Warm Springs Tribes
Toy et al. (1996)	NA	NA	NA	53 (median, males) 34 (median, females)	Surveyed members of the Tulalip Tribe
				66 (median, males) 25 (median, females)	Surveyed members of the Squaxin Island Tribe

Note. Consumption rates (CR) from various populations used to calculate the Screening values for arsenic and lead suggested by EPA. Source: U.S. EPA 2000

1.4.5) Quinault Indian Nation Diet and Significance of Shellfish

Washington is home to a wide range of water resources that support commercial, recreational, and subsistence fishing and harvesting. Of the many Washington residents that consume local fish and shellfish, members of Native American tribal nations have been identified as consuming larger amounts of finfish and shellfish than the general population (EPA 1995). Among the federally recognized and non-federally recognized Native American tribes, the Quinault Indian Nation has an intertwined culture and economy in connection with the natural environment and its resources (Quinaultindiannation.com). This includes the Quinault Pride, a seafood processing facility that purchases the majority of tribal harvest (Crosman et al. 2019). The Quinault Indian Nation (QIN) is a federally recognized, self-regulating sovereign nation on the central pacific coast of Washington State with treaty rights to numerous fish and shellfish species. It consists of members of descendants of seven coastal tribes including the Quinault, Queets, Quileute, Hoh, Chehalis, Chinook, and the Cowlitz (Quinaultindiannation.com). Shellfish have been a mainstay of the tribes of the QIN for thousands of years. Clams, crab, and many other species are readily available for harvest. The rapid decline of many western Washington salmon stocks, due in large to habitat loss, has pushed shellfish to the forefront of many tribal economies (Northwest Indian Fisheries Commission). As with salmon, the right to harvest shellfish lies within a series of treaties signed with representatives of the federal government in the 1850's. Among the shellfish harvested, Dungeness crab is potentially the most lucrative economically. Commercial crab fishing was an estimated $86 billion industry in Washington state in 2022 (Hart 2023). In addition, 28.7 million pounds of Dungeness crabs from 2022-2023 alone were taken from Washington Coast (WDFW 2024). Aside from the commercial uses, Dungeness crabs represent a vital subsistence in home uses as well as an important point of cultural association for the tribe's identity (Donatuto et al.2003). Dungeness crabs are employed in cultural ceremonies,

incorporated in common diet, and sold to support families on and off the reservation. Due to their high- protein diet of subsistence harvested foods such as Dungeness crabs, the QIN may be at risk for heath related problems with the prevalence of bioaccumulative pollutants such as lead and arsenic. Low level chronic exposure to lead and arsenic could result in adverse effects on the tribe's population who depend on that food source. Therefore, we speculate that the compounding effects of the possible contamination of these shellfish threatens not only their health and economical livelihood, but also their mental, social, and spiritual health.

1.5) Bioaccumulation of Heavy Metals in Marine Organisms

Heavy metals discharged into the aquatic systems by natural or anthropogenic sources during transport can become bioavailable for uptake by aquatic organisms. Different kinds of uptake occur, at all trophic levels, whether the metals are essential or not (Gaulier et al.2019). For example, primary producers such as phytoplankton, zooplankton, and algae take up inorganic arsenic from seawater (Chen et al. 2000) and convert it via several biosynthetic steps to the water-soluble arsenosugars and fat-soluble arsenophospholids that lead to the formation of the end product arsenobetaine, the dominant organoarsenic compound in marine organisms (Francesconi et al. 1996). Subsequently, the consumption of these organisms results in the passage of heavy metals through the food chain (Ahlf et al.2009). Due to

Heavy metals not being chemically biodegradable, they can accumulate to a certain extent in the tissues of many marine species in a process called bioaccumulation (Gaulier et al.2019).

For higher organisms, heavy metals can be incorporated through diet, through respiratory pathways via gills, and via environmental exposure (Jakimska et al. 2011). Accumulated metals in organisms are bound to metal binding proteins and either stored in cells, tissues, or are eliminated (excretion via feces, eggs, molts) (Rumisha et al. 2017). The subsequent fate of heavy

metals depends on the physiology of the organism, as to whether the metal is used for an essential metabolic purpose, excreted, or stored in the body (Rainbow et al. 2002). Additionally, factors such as species type, body size, age of organism, ingestion rate, gut passage time, and presence of iron oxides can all potentially influence arsenic and lead absorption (Zhang et al. 2022).

1.5.1) Heavy Metal Accumulation in Crustaceans:

Crustaceans have been identified among marine organisms to bioaccumulate high concentrations of heavy metals from food, water and/or sediment (Reichmuth et al. 2010). In particular, the five main routes are: via food or non-food particles, gills, oral consumption of water, and the skin (Zaman 2013).

Crustaceans can accumulate heavy metals in their dissolved form through the use their gills to osmoregulate (Henry et al. 2012. This process involves letting water through the surface tissue gradient to facilitate the exchange of its vital respiratory gases and inorganic osmolytes such as salts (Engel et al. 1979). As a result, the gills are susceptible to bioaccumulating metals over time due to the constant contact of water movement through this tissue (Shah et al. 2020). Additionally, the absorbed heavy metal ions through the gills are transported throughout the various body parts through blood, resulting in accumulation in surface cells such as the muscle tissue (Liu et al. 2023).

Recent studies suggest that diet, however, may be the major source of metals for many marine crustaceans (Wang 2002). As mentioned in a previous section (see section 1.3.1), crustaceans may accumulate heavy metals through trophic transfer by consuming other organisms that are contaminated thus transferring a portion of heavy metal accumulates in their gut membrane. Crustaceans have a wide variety of eating habits and diet among their numerous

species. In most cases, crustaceans are omnivorous scavengers that feed on organic matter left be larger predators, as well as some crustaceans are predators that hunt and eat small fish, microscopic organisms, algae, plankton, snails, plants, eggs of other marine life, mussels, worms and even other crustaceans (Schubiger 2022). Among other food sources, Crustaceans that are deposit feeders can accumulate heavy metals directly from the contaminated sediments through ingesting sediment as a food source to sort organic matter and detritus, as well as through constructing underground burrows (Siddiqui et al 2021).

The hepatopancreas serves as a critical organ for crustaceans in the absorption and storage of nutrients, detoxifying foreign substances such as heavy metals, and synthesizing digestive enzymes for food digestion (Wang et al.2014). The stored nutrients can be transported to the muscle, gonads and other tissues during the growth and reproductive stages. Some crustaceans can store large amounts of energy, particularly lipids, in the hepatopancreas to be used for energy expenditure during molting, starvation, or reproduction. Metabolically active tissues such as the hepatopancreas and gills have been identified to contain higher heavy metal accumulation compared to the muscle tissue in crustaceans. For example, the hepatopancreas has been found to only account for 5 % of the body weight in crustaceans, while containing 75-95% of heavy metals accumulated throughout the crustacean's muscle and organs (Hopkin et al. 1982). Overall, heavy metals taken up by the crustacean (from dissolved form and food sources) will enter in a form that is initially available to bind with the metabolites in the cells, with the potential to be transported elsewhere in the body via the haemolyph (Mardsen et al. 2004). These metals then have the potential to play a role in the metabolism of the crustacean such as essential metals fulfilling metabolic functions, or a toxic role by binding in the wrong place.

1.5.2) Crustaceans as Bioindicators.

Pollution monitoring is the protection of ecosystems and human beings, with the main interest in the use of quantitative sentinel organisms with regard to water or sediment, is their capacity to give information on the bioavailability of pollutants (Richir et al. 2016). Recently, active monitoring approaches are based on organisms that were developed with the aim to solve some limitations of passive methodologies by including the effective presence of native organisms in sampling sites (Besse et al.2012). A bioindicator is an organism that allows by reference to biochemical, cytological, physiological, ecological or ethological variables, in a practical and safe way to characterize the status of an ecosystem, and to highlight as early as possible their changes in the environment (Richir et al. 2016). Biomonitors can establish geographical and/or temporal variations in the bioavailable concentrations of pollutants metals in coastal and estuarine waters by providing a quantitative aspect of the environment (Rainbow et al. 1993; MacFarlane et al. 2000). Furthermore, an effective biological indicator must reflect levels of environmental contamination, the relationship remains constant spatially and temporally, and they should not regulate the total concentration of contaminants in its body tissues (Depledge et al. 1994; Rainbow 1993). Additionally, bio-indicators should be relatively sedentary or resident to the area of interest, easy to identify, abundant, long lived, be available for sampling all year and have a wide distribution (Richer et al.2016). They should also be tolerant of exposure to environmental variations in physicochemical parameters and provide sufficient tissue for individual analysis (Rainbow 1993).

Decapod crustaceans are very common and have a widespread distribution in different habitats (e.g soft and hard bottom substrate from intertidal to deep environments) and geographical areas (Navarro-Barranco et al. 2020). They have a wide range of eating habits including being scavengers, omnivores, predators, and deposit feeders. Crustaceans may be

especially sensitive to pollution and other types of habitat degradation because they reside in bottom sediments where chemical contaminates accumulate (Turkmen et al.2006). They also represent an important link in the trophic web, considering that all stages in the crustacean's life cycle make them a relevant food component for other species, thus playing a major role in the transference of pollutants to higher trophic levels (Simmonetti et al. 2012). Overall, these adaptations provide numerous pathways for bioaccumulation of heavy metals, from absorption of the gill surface, ingestion of water and sediment, and through the consumption of previously accumulated pollutants in other organisms (MacFarlane et al. 2000), thus making crustaceans excellent candidates as bioindicators and biomonitoring programs.

1.5.2.1) Dungeness Crabs

Metacarcinus magister (synonym: *Cancer magister*) commonly referred to as Dungeness crab, are distinctly identified by their oval shaped carapace, which is yellow- brown to purplish in color, as well as by the fact their claws have light-colored tips, sharp serrated teeth, and pronounced hooks at the tips, distinguishing it from similar species (Canadian Fisheries 2016). They are found in ranges from the Aleutian Islands, Alaska, to northern Mexico in the eastern Pacific (Rasmuson et al. 2013). Adults inhabit sandy or muddy bottoms and eelgrass beds in bays, inlets, estuaries, and on the open water at depths ranging from the intertidal zone to about 750 feet, while juveniles frequent shallow estuarine areas with protective structures such as pilings or woody debris and avoid habitat with adult crabs (Canadian Fisheries 2016). Adults will bury themselves in sandy and mud bottoms during the day, and commonly emerge from the substrate during nocturnal high tides (Mcgaw 2005). Dungeness crabs are an important prey item in all life history stages (Rasmuson et al. 2013). For example, adults and juveniles are eaten by a variety of fish and mammals such as harbor seals and sealions. Their larvae are an important

food source for pacific herring, rockfish, and salmon. M. *magister* are opportunistic omnivores and scavengers, feeding on a variety of small invertebrates, fish, and even other crabs (Jamieson et al. 1990). In estuaries, many populations move in and out of the intertidal each day to forage typically under the cover of darkness, so crabs can avoid visual predators (Holsman et al. 2006). They are also cannibalistic, and adults will feed on juvenile crabs during their first year, as well as juvenile crabs being highly cannibalistic during their first year; thus, first year cohorts are strongly influenced by survival of later cohorts (Fernandez et al. 1993). Maximum life expectancy is 8 to 13 years, but commercial caught individuals are usually about 4 years old (Rasmuson et al. 2013).

1.5.3) Toxicity of Heavy Metals to Crustaceans

Heavy metal bioaccumulation in marine biota can produce harmful impacts on crustaceans, at higher exposure levels; however, some of these heavy metals have a relatively high density and can be toxic in low quantities, such as lead and arsenic (Govind 2014). The excess quantities of these heavy metals can elicit toxic effects such as the interference in the biochemical role of metabolic processes and even resulting in death (Jakimska et al.2011). Crustaceans, however, can detoxify or excrete these heavy metals to avoid potential adverse effects (Rainbow 2002). Detoxification requires the metal to be bound with such a high infinity that it is unavailable to be bound to other metabolites, thereby preventing these latter from completing their metabolic role (Marsden et al. 2004). So long as the combined rates of detoxification and excretion exceed the rate of metal uptake, the incoming metal will not have a toxic effect on the crustacean. If the rate of uptake exceeds the maximum combined rate of detoxification plus excretion, then the concentration of metabolically available metal increases and may reach a threshold at which sublethal and finally lethal toxic effects are exhibited (Rainbow 2002). At a molecular

biochemical and cellular level, the mechanisms of heavy metal toxicity in crustaceans are similar to those of humans (see Section 1.3). High concentrations of metals in crustaceans can also hinder ecologically important processes such as the impairment of growth, development, molt cycle, limb regeneration, biochemistry, and physiology survival (Reichmuth et al. 2010).

Lead can affect crustacean's nervous system by accumulating in the brain and inhibiting sulfhydryl groups containing enzymes thus affecting cell membranes (Jacobson and Turner 1980). Along with lead inhibiting enzymes, it has also been found to alter mitochondrial function and delay mitosis due to the disruption of mitotic spindle (Chin et al. 1978). With respect to the cell membrane itself, lead is thought to inhibit membrane bound enzymes such as Na^+/ K^+ ATPhase and to cause oxidative destruction of polyunsaturated lipids present in the membrane (Fingermen et al.1996).

Heavy metals can affect the endocrine system in crustaceans by changing their hormone levels that can result in fluctuations in key physiological processes such as molting, limb regeneration, blood glucose level, color changes, and reproduction (Fingermen et al.1996). For example, typically, after a crab loses a limb, the regenerating limb develops within a layer of cuticle and unfold at ecdysis when it becomes functional (Fingermen et al.1996), however, Weis et al. (1992) found that lead can retard limb regeneration and molting of U.*pugilator* (fiddler crab). Limb generation and molting are closely related to each other in crustaceans. The growth of crustaceans is also regulated by molting, which is the process of shedding their old exoskeleton and synthesizing a new one (Hosamani et al. 2017). Another example of the effects of heavy metals on the endocrine system is hyperglycemia, anemia, and depletion of plasma ions, which can typically occur in crustaceans during sublethal exposure to metals. For instance, Reddy et al. (1994) found that heavy metals not only induce hyperglycemia in crustaceans such

as in crayfish, by causing crustaceans' hyperglycemic hormone (CHH) release, but they also inhibit CHH synthesis. Color changes in crustaceans are regulated by pigment dispersing and pigment concentrating neurohormones that distribute within the chromatophores (Fingerman et al. 1996). Reddy and Fingermen (1995) found that heavy metals can affect the coloration of U.*pugilator* (fiddler crab) by inhibiting the release of black pigment dispersing hormone (BPDH), thus leading to lighter colored individuals vs unexposed ones.

In terms of reproduction, crustaceans have two acting neurohormones that play key roles in the regulation of gonadal maturation (Fingermen et al.1996). One is the gonad inhibiting hormone (GIH) from the sinus gland and the other is the gonad stimulating hormone (GSH) found in the brain and thoracic ganglia. Heavy metals have been found to effect the reproduction of crustaceans by reducing the percentage of viable hatchlings per brood, changing the age at first reproduction, and reduction in body size (Ravera and Gatti 1988). Additionally, lead and arsenic can affect the reproduction of crustaceans through the interferences with the ovarian cycles such as in Carcinus *maenas*, by significantly reducing alkaline phosphates and reducing ovarian protein content through tissue destruction, disturbance of cellular function, and impairment of protein synthesis (Elumalai et al. 2005). Overall, these are a few examples highlighting the necessity of analyzing the destructive nature of heavy metals at sublethal to lethal levels in crustacean populations that could possibly be discovered in the natural environment.

1.5.4) Environmental Factors Affecting Accumulation of Heavy Metals

The ability for heavy metals such as As and Pb to become bioavailable to crustaceans and accumulate is influenced by the speciation of the metals, especially in terms of separating between the water and sediment (Ansari et al. 2004).Bioavailability refers to the portion of the

total quantity or concentration of chemical that is potentially available for uptake by aquatic organisms, while speciation refers to the various physical and chemical forms in which an element may exist in the system (Ansari et al.2004). In general, Crustaceans accumulate both organic and inorganic forms of As (Taylor et al. 2017). Inorganic arsenic (iAs) predominates in seawater and sediments, however crustaceans bioaccumulate the element typically as organic compounds (Francesconi et al. 1998) by converting the iAs into organic As such as arsenobetaine (AsB), arsenocholine (AsC), momomethylarsenic acid (MMA), and dimethylarsenic acid (DMA) (Ghosh et al. 2022). Variations can be found in different concentrations and the distribution of arsenic's chemical form in marine organisms, often reflecting their trophic position, and the capability of transforming different forms of arsenic or other species-specific traits in metabolizing As (Fattorini et al. 2004).

Along with the speciation of heavy metals affecting accumulation rates in crustaceans, bioavailability of the metals is influenced by a complex variety of interrelated factors; both physio-chemical properties of metals, sediments, water, and biological characteristics of the organisms involved. Organism's biological characteristics include species type, size, sex, reproductive cycle, feeding habits, movement patterns, and age (Ali et al.2019; Zaynab et al. 2022). For example, higher concentrations of arsenic appear to be present in tissues of crustaceans that feed primarily on phytoplankton or macroalgae, which often contains higher concentrations of inorganic and organic arsenic (Neff 1997). Environmental factors such as pH, salinity, dissolved organic matter (DOM) levels, hardness, and temperature all potentially influence heavy metal absorption by crustaceans as well (Zhang et al. 2022)(Table 3). Salinity can influence heavy metal bioavailability by changing the geochemistry of metals and physiological attributes of aquatic organisms. For example, Karar et al. (2019) found that the

blue swimmer crab (*Portunus pelagicus*) had higher accumulation levels of lead in the hepatopancreas and muscle tissue during the monsoon season (lower salinity) vs non-monsoon season in Bengal, India. Arsenic uptake rates, however, have been found to be positively correlated with higher salinity environments in *Metapenaeopsis palmensis* and *L.vannamei* (Zhang et el. 2018; Valentino-Alvarez et al. 2013). Several studies have observed that when crustaceans have been exposed to lower salinities (below their isosmotic point) a higher accumulation of heavy metals such as arsenic, occurs (Fowler et al. 1978). This was speculated to be related to an increase in water uptake promoted by exposure to a hypo-osmotic environment that favors dissolved toxic diffusion into the cells (Grosell et al.2007). In addition, higher osmoregulation mechanisms increase water excretion, as well as the capture of major ions (Cl^-, Na^+) that can be replaced by toxic elements. These examples overall show different uptakes rates with variable salinity conditions depends on the physicochemical form of the metal present, but may, in some cases, also be affected by the physiological responses in particular osmoregulation processes, by the crustaceans itself (Rainbow 1997b). This could be evident when Bryant et al. (1985) found that the crustacean *Corphium volutator,* when subjected to variations in salinity, had no significant effect on arsenic uptake in the organism and attributed this to the crustacean being adapted to temperate estuarine waters, where there is wide range of salinity conditions.

Typically, environmental temperature can affect the bioavailability of metals due to the higher solubility of metal compounds with increased temperature, and thus higher concentrations of free metal ions (most bioavailable form) (Sokolova et al.2008). Also, an increase in metabolic rates at elevated temperatures may contribute to metal accumulation in crustaceans, due to a higher energy demand (Sokolova et al.2008). This higher energy demand results in elevated

ventilation and/or feeding rates, which in turn could lead to higher exposure to metal contaminated water or food (Willmer et al. 2000).

Alterations in seawater chemistry such as lower pH can affect the solubility, speciation, and distribution of heavy metals in sediments and water, potentially affecting the toxicity of these metals in crustaceans (Ivanina et al. 2015). Low pH increases the solubility of heavy metals and can cause metal desorption from the sediments and organic ligands, resulting in a higher influx of dissolved metals into the water column. Therefore, it can create a higher probability of accumulation in crustaceans (Ivanina et al. 2015). For example, although Adeleke et al. (2020) found that accumulation rates of Pb (lead) in the muscle tissue of the crab *Dotilla Fenestrata* when exposed to varying pH conditions did not follow a defined pattern with increasing or decreasing pH, they did find Pb accumulation rates were more dependent on the bioavailability levels in the environment as well as the regulatory physiology of the organism itself. This was speculated to be possibly due to Pb's ability to form significant bonds with both Cl^- and CO_3^{2-}; therefore, observing that as pH decreases, the free ion form of Pb increases, resulting in a great increase in its bonding with Cl^- (Millero et al.2009).

Overall, variables in marine systems such as pH and salinity can play a pertinent role in determining the chemical speciation of heavy metals and the toxicity associated with them in two different ways (Riba et al. 2003). First, by affecting the chemical speciation of metals producing more bioavailable species at lower values of pH and salinity. Secondly, by influencing the sensitivity of organisms in requiring higher metabolic rates and ventilation. However, as previously stated, there is a wide range of complexity in the intercorrelated factors such as organismal characteristics, physio-chemical properties of metals, sediments, and water, as well as environmental factors that potentially facilitate the bioaccumulation process in crustaceans.

Table 3.

Environmental Parameters Effects on Heavy Metal Accumulation

Environmental Parameter	Heavy Metal Accumulation Rates	Explanation
Lower pH	Increases	Increases solubility of metals, metal desorption from sediments and organic matter, resulting in dissolved metals in water column
High/low salinity	Increases or decreases	Induces physiological responses(osmoregulatory) in a particular species dependent on their isosmotic point.
Increase temperature	Increases	Increases metabolic rates/higher energy demand in organisms resulting in elevated ventilation and/or feeding rates.

Note. Environmental conditions that may attribute to increases or decreases in heavy metal accumulation in marine organisms.

1.5.5) Coastal and Urban Trends of Pb and As Accumulation

Information on determining the geographic extent and magnitude of arsenic and lead in Dungeness crabs across varying marine waters throughout Washington state is limited. However, the few studies that have used this organism as a bioindicator have found significant results in terms of reporting lead and arsenic concentrations that may be a human health risk (Table 4). For instance, Johnson et al. (2000) found a human health risk in the detectable levels of arsenic in Dungeness crabs caught within the Puget Sound (Padilla Bay, Fidalgo bay, Hat Island) ranging

from 5230- 8390 µg/Kg as well as lead ranging from 20- 29 µg/Kg, however the results did not indicate the chemical speciation of arsenic that was present. In contrast, lead concentrations were considered as background levels for the Puget Sound and not posing a significant human health threat for tribal or recreational consumption of these shellfish. The study did not identify sources that could be affecting these metal concentrations throughout the study area. The potential sources that were listed such as in Fidalgo Bay (Crandall Spit) and Pidilla Bay (March Point) were attributed to wastewater treatment plant discharges, stormwater, road runoff, and refinery effluents, but they did not actually measure concentrations in these direct areas. Overall, though they attributed the concentrations of heavy metals to urban influence.

Johnson et al. (2002) furthered their analysis in determining the geographic range of arsenic contamination by examining Dungeness crabs caught in Sinclair Inlet, Port Orchard, Eagle Harbor, and Dyes Inlet, WA and found total arsenic concentrations ranging between 3.8 – 5.0 µg/Kg (wet weight). This time, however, they tested for the specific Inorganic arsenic form, which is more toxic than organic forms, and found it ranged from 0.47- 44 µg/Kg thus accounting for 0.2% or less of the total arsenic observed. In addition, the inorganic arsenic concentrations were below the threshold for being considered unhealthy for human consumption and considered as natural conditions in the Puget Sound.

In 2011, Washington State Department of Fish and Wildlife (WDFW) evaluated the magnitude of toxic pollutants in Dungeness crabs throughout the Puget Sound and found organic arsenic levels ranged from 3440 µg/Kg wet weight in Commencement Bay to 20,500 µg/Kg in Marine Area 8.2 (Port Susan and Port Gardner) (Carey et al.2014). Lead, however, was only detected in 39.2 % of Dungeness crab muscle, and means ranged from 3.8 µg/Kg wet weight in Marine Area 8.2 to 23 µg/Kg in Commencement Bay. Overall, they found concentrations of

heavy metals were highly variable between Marine Areas and showed no pattern between metals and urban locations, thus suggesting the relation between human activities and metal concentrations were unrelated or that Dungeness crabs can metabolically regulate metals, resulting in little accumulation in muscle tissue.

A similar study sampling Dungeness crabs in Port Angeles harbor, WA, near the Rayonier Mill, found arsenic levels at median concentrations of 5,850 µg/kg (wet weight), but indicated that individuals consuming these organisms would not result in producing adverse health effects (Langmann 1999). However, the study suggested their smaller sample size, and possibility of not sampling in areas where the highest levels of contamination deposition could have occurred, related some level of uncertainty and the necessity for further examination of the area. Sources of contamination were identified to be the operation of the recently decommissioned Rayonier pulp mill. This included the spilling of hydraulic fluid and other tank leaks/spills into soils, groundwater, and the nearby Ennis Creek over the years of operation.

Within Bellingham Bay, WA, Cubbage et al. (1991) investigated the potential bioaccumulation of contaminates including arsenic and lead in Dungeness crabs. Arsenic concentrations ranged from 1,900 - 5600 µg/kg, with the highest concentrations in the crustaceans found occurring on the west side of the bay, farthest away from Bellingham. Lead values ranged from non-detectable - 290 µg/kg. Both arsenic and lead levels were considered well under the required guidelines by the U.S Food and Drug Administration (FDA) for the potential transfer of harmful chemicals to humans via seafood consumption. Thus, overall, they interpreted the concentrations of metals in Bellingham Bay to be low compared to areas with known sediment contamination and equivalent to background levels within the area. In a concurrent study in 1991 performed by the Science Applications International Corporation

(SAIC) at the Puget Sound Dredged Disposal Analysis (PSDDA) site in the middle of Bellingham Bay, showed equivalent concentrations of lead found in the crab muscle compared to Cubbage et al. (1990), while arsenic levels were considerably lower.

Overall, this literature generally measures total arsenic and total lead concentrations, and show that while variations of As and Pb found in Dungeness crabs do vary by location, the majority of the time, concentrations are below levels that would negatively affect human health. The exception is from assessing Padilla Bay, Fidalgo bay, Hat Island (Johnson et al (2000), however as mentioned earlier, they did not indicate the chemical speciation of arsenic that was present. In addition, these studies were able to identify different possible sources of contamination, whether it be due to industrial impacts, storm water runoff, domestic wastewater, a combination of urban influence, or no suggestions for the sources of contamination within these urbanized areas. Due to this previous literature, we have some points of reference to compare concentrations of arsenic and lead throughout the Puget Sound and urbanized marine areas within Washington State. However, previous literature that includes study sites located in more rural areas, such as along the Washington coast are lacking, thus leaving large gaps in determining risk assessments for the consumption of these organisms caught within this environment. With the combination of a significant amount of Dungeness crabs being taken from the Washington Coast per year, 28.7 million pounds from 2022-2023 (WDFW 2024), and the lack of information on possible contamination of the area, this poses a possible human health threat in itself.

Another marine area within Washington state that has been largely unexplored is the Hood Canal. This natural fjord is located to the west of main part of the Puget Sound, and although connected to the Puget sound, can be identified as its own unique marine boundary

(Encyclopedia of Puget Sound). Carey et al. (2014), in addition to evaluating the Puget Sound, also evaluated the magnitude of arsenic and lead in Dungeness crab within Hood Canal. Similar to the Puget Sound, arsenic concentrations ranged between 0 to 10,000 ug/kg, and lead concentration ranged between non detectable to 0.0041 µg/kg. Both concentration values were considered below levels that would negatively affect human health. An explanation for this accumulation pattern could be attributed to the Hood Canal region being less developed than other Puget Sound Basins (Long et al. 2010). Most of the shoreline is sparsely populated with individual homes, rental properties, and resorts; however, an estimated 33 percent of the shoreline is still modified by human activity (WA USGS). Furthermore, potential anthropogenic sources of heavy metal contamination to the canal include many small marinas, several small towns and villages, farms, a bordering highway, a navy submarine base, and stormwater runoff entering via the tributary rivers and streams (Long et al. 2010). Also, this area is unique in that historically, the shape and geography of the Hood Canal produces poor water circulation in turn producing low dissolved oxygen (DO) (Newton et al. 2007) Low DO values have been identified to increase the levels of heavy metals in sediment (Surbakti et al. 2021). Therefore, although higher accumulation patterns were not observed by Carey et al (2014), we speculate that the combination of environmental parameters and anthropogenic pressures contributing to this unique marine ecosystem, could lead to higher levels of heavy metal contamination, thus pertaining to the necessity for examining possible heavy metal contamination in this watershed.

Table 4.

Metal Concentrations Reported in Studies on Puget Sound

Reference	Location	Arsenic Range (ug/Kg)	Lead Range (ug/Kg)	Health standard-As	Health standard-Pb	Influences on accumulation pattern
Johnson et al. 2000	Fidalgo Bay, Padilla Bay, Hat Island	5230- 8390 (organic)	20 -29	Above	Below	Wastewater treatment plant discharge, stormwater runoff, refinery effluents.
Johnson et al. 2002	Sinclair inlet, Eagle Harbor, Dyes Inlet	0.47 - 44 (inorganic)	NA	Below	N/A	Urban influence
Carey et al. 2014	Commencement Bay, Port Susan, Port Gardner, Elliott Bay	3440- 20,500 (organic)	20 -29	Below	Below	Urban influences observed in Pb concentrations, but no direct relationship between urban locations and As concentrations.
Langman 1999	Port Angeles	5230-8,390 (organic)	3.8 - 23	Below	Below	Operation of Rayonier pulp mill
Cubbage 1991	Bellingham Bay	1,900 - 5600 (organic)	N/D - 290	Below	Below	No observed relationship between urban geographical area and heavy metal contamination.

Note. Arsenic and lead concentrations found in previous studies on Puget Sound Dungeness crab tissues (ug/kg), wet weight. N/D = not detectable.

1.6) Heavy Metal Quantification

The negative effects of pollutants, such as heavy metals, are exerted at different levels of biological organization and at different timescales (Lionetto et al. 2019). The growing concern towards the harmful effects of heavy metals on wildlife and human health accounts for the growing importance for early warning tools for identification, estimation, and assessment of the risks posed by these pollutants discharged into the environment (Lionetto et al. 2019), which include the specifications of localized environmental conditions and portraying the real effects of exposing living organisms to these heavy metals (Clasen et al. 2022). As mentioned in section

1.4.2, biomonitoring efforts can identify chemicals found in the environment and monitor trends as well as the distribution of exposure in the aquatic ecosystems (Richir et al. 2016). Environmental measures involving various biological samples such as air, water, soil, and organisms that can be used to detect the presence of hazards and assessing their relative severity. Scientist have traditionally conducted chemical assays and directly measured physical parameters of the environment such as temperature, salinity, nutrients, pollutants, gas levels, whereas the use of bioindicators use organisms to assess the cumulative impacts of both chemical pollutants and habitat alterations over time (Scott et al. 2010). Consequently, the use of bioindicators is fundamentally different from classic measures of environmental quality and offers some advantages. Bioindicators add a temporal component corresponding to the life span or the residence time of an organism in a particular system, thus allowing the integration of a current, past and future environmental conditions, while some traditional chemical measurements only represent conditions found at the time of sampling (Markert et al. 2003). Secondly, using bioindicators can reveal indirect biotic effects of pollutants when many traditional physical or chemical measurements cannot. Indirect contaminant effects can be difficult to gather from chemical measurements such as in the case of bioaccumulation (Scott et al.2010). Lastly, with thousands of substances and factors to monitor, scientist understand that biota itself are the best predictors of how ecosystems respond to disturbance or presence of stressors. A common problem with traditional chemical and physical measurements is that they simplify a complicated response inherent in species rich habitats, while using bioindicators rely upon the complicated intricacies of ecosystems and are more representative of the overall dynamic condition of the environment. Therefore, biomonitors can be valuable for an integrated approach addressed to circumvent strategies for prevention or reduction of deleterious health effects of chemical

contamination such as heavy metals, in the environment as well as in humans (Lionnetto et al. 2019).

1.6.1) Acid Digestion of Suspended Particulate Matter (SPM)

In terms of quantifying heavy metal accumulation in the environment, sample introduction for the analysis of gaseous, liquid, and solid samples using analytical instruments are often required to be in a liquid form (Gaulier 2019). This requires either digesting solid samples using a mixture of strong acids such as nitric acid (HNO3), Hydrofluoric acid (HF), hydrochloric acid (HCl), because of their strong oxidizing ability, as well as filtering suspended particulate matter (SPM), and acidifying samples (Mohammed et al. 2017). Nitric acid is the most commonly used acid for oxidation of organic matrices with its oxidizing strength being increased when used in conjunction with other acids or chlorate, permanganate and hydrogen peroxide (Mohammed et al. 2017). Depending on the expected concentrations of the elements of concern and/or chemical composition (for example, very acidified samples), the liquid samples can be diluted prior to analysis, in order to protect the instrument's tubing and/or to be consistent with the calibration range (Gaulier 2019). Various points should be taken into consideration regarding sample handling and preparation. Many instruments only use a few mL of samples, so care must be taken to ensure that the collected samples are representative of the bulk material (Raja et al. 2019). Also, contamination prior to measurements constitutes a serious concern, especially when the targeted analytes are expected to be present at very low concentrations (Gaulier 2019). The resulted liquid samples through these procedures are finally stored, diluted, and analyzed using several analytical instruments depending on the elements of interest and demanding limits of detection (Gaulier 2019).

1.6.2) Inductively Coupled Plasma- Mass Spectrometry (ICP-MS)

Instrumental analytical methods may be employed to measure the concentrations levels of heavy metals in various samples. Furthermore, they can identify the elemental speciation in samples which has direct influence on their toxicity, bioavailability, and environmental impact (Ray et al. 2004). This includes the inductively coupled plasma mass spectrometry (ICP-MS). This quantitative multi-element measuring systems offers a wide detection range of elements (Helalluddin et al.2016) as well as permits simultaneous analysis of multiple elements (Ray et al. 2004). Sample preparation for the ICP-MS is relatively simple. The main requirement is that samples must be in a liquid form. Liquid biological samples are usually diluted or as previously stated (see section 1.5.1), solid samples are digested using a mixture of strong acids such as nitric acid (HNO3), as well as filtering suspended particulate matter (SPM) (Mohammed et al. 2017). In the sample, a total dissolved solid (TDS) content of <0.2 % (2g/L) is also recommended in the ICP-MS to reduce sample- specific matrix effects and the potential for nebulizer blockage (Wilschefski et al. 2019). The ICP-MS uses an argon plasma source to dissociate the sample into its basic atoms or ions (Helalluddin et al.2016). The ions are released from the plasma and funneled into the mass spectrometer, where they are isolated according to their atomic mass-to-charge ratio by a quadrupole mass analyzer. This methodology thus detects metal ions rather than the light that they emit. Due to the ICP-MS completely atomizing the sample, different chemical forms of an element are indistinguishable after the sample reaches the plasma. Thus, if different forms of the element are sought (organic vs inorganic), chromatographic systems such as ion-exchange HPLC or gas chromatography can be coupled with the ICP-MS by connecting the end of the analytical column to the nebulizer with a capillary tube (Wilschefski et al. 2019). This effectively allows the different species of an element to be effectively separated before the sample reaches the plasma and measured individually. However, in the terms of this study, the

ICP-MS alone was used, therefore is only able to identify total organic elemental concentrations. Overall, the ICP-MS has many benefits, providing low detection limits, normally in the part per trillion (ppt), low background detection, multielement capability, short analysis time, and simple sample preparation (Brown et al. 2005;Ray et al. 2004). Therefore, it offers the opportunity for high quality assessment of arsenic and lead in the laboratory.

1.7) Conclusion

Lead and arsenic are heavy metals that are considered non-essential to any biological organism and can cause negative effects in organisms at lower concentrations (Rainbow 2002). These metals are introduced into marine ecosystems through various natural and anthropogenic sources (Saleh et al. 2018). However, anthropogenic sources such as industrial wastes, stormwater run-off, and municipal wastes have increased over the generations leading to high levels of heavy metal contamination through marine ecosystems (Akter et al.2005). Once these heavy metals are discharged into marine ecosystems, they remain in the ecosystem by dissolving into metal ions in the water (aqueous phase) or are accumulated as particulate matter in sediments (Smedley et al.2002). Typically, lead ions are absorbed by sediments (Denton et al.1997), while arsenic can remain in its dissolved form and remain mobile under a wide range of environmental conditions (Smedley et al.2002). Both these heavy metals go through various series of transformations leading to a wide range of organic and inorganic compound species found in marine ecosystems (O'Neil 1998; Botte et al. 2022; Smedley et al. 2002). Crustaceans are among the marine organisms that are able to bioaccumulate lead and arsenic through water, sediment, and food in organic and inorganic forms (Reichmuth et al. 2010) thus being used as bioindicators for determining heavy metal contamination in marine ecosystems (MacFarlane et al. 2000). The bioavailability of these heavy metals to be accumulated depends on the chemical speciation,

which can be affected by physio-chemical properties of the metals, sediment, water, and physiological characteristics of the organism. Arsenic and lead accumulation in crustaceans can be detrimental to their key biological processes (Govind 2014; Reichmuth et al. 2010) and can transfer accumulates to humans through seafood consumption, where they can elicit harmful effects to human health (Liu et al. 2019). In shellfish, lead is considered toxic to humans whether it's in the organic or inorganic forms (Liu et al. 2019; Assi et al. 2016). Arsenic in shellfish, however, is primarily in the organic form as arsenobetaine, which is considered nontoxic to humans (Ballin et al.1994). The toxic species of most concern are inorganic arsenic, and methylated organic arsenic forms such as monomethylarsenic acid(MMA), and dimethlyarsenic (DMA) (Johnson et al.2002). Dungeness crabs are a highly consumed and economically important species to tribal and non-tribal communities throughout Washington State (Hart 2023; Donatuto et al.2003). Therefore, testing the accumulation of Dungeness crabs from different marine environments throughout Washington state is necessary for determining the health risk in consuming these species. Therefore, this project will provide data on the levels of arsenic and lead accumulation in Dungeness crabs caught from the Puget Sound (Hood Canal) and Washington coast to evaluate if consuming them constitutes a human health risk.

Methods

Sample Collection

To address the research questions outlined above, two sites were chosen for the field sampling of Dungeness crabs within the U.S. State of Washington. One site was sampled within the Puget Sound (at the Northern entrance to the Hood Canal within the Western side of the Puget Sound), whereas the other occurred along the outer coast of Washington State. The samples collected within Hood Canal consisted of 15 Dungeness crabs obtained from the Port Gamble S'Klallam tribe (Figure 1). These crabs were caught between December 6 – 7 primarily using standard fishing gear (baited pots) at shallow depths between 0-300 meters. Only Dungeness crabs that met standards from the Washington Department of Fish and Wildlife (WDFW) fishing regulations were used in the study. This included hard-shelled male Dungeness crabs measuring a width of greater than 6.25 inches across the widest part of the carapace (shell), between the notches in front of the largest lateral spines (WDFW 2019). At the time of purchasing, individual Dungeness crabs were placed in gallon-sized Ziplock bags and placed in offsite freezers to prevent contamination and preserve the specimen. The organisms were then transported in coolers filled with enough ice to prevent thawing to the Evergreen State College labs, where they were removed from the coolers and preserved in a -15^{o}C – 20^{o}C freezer for further analysis.

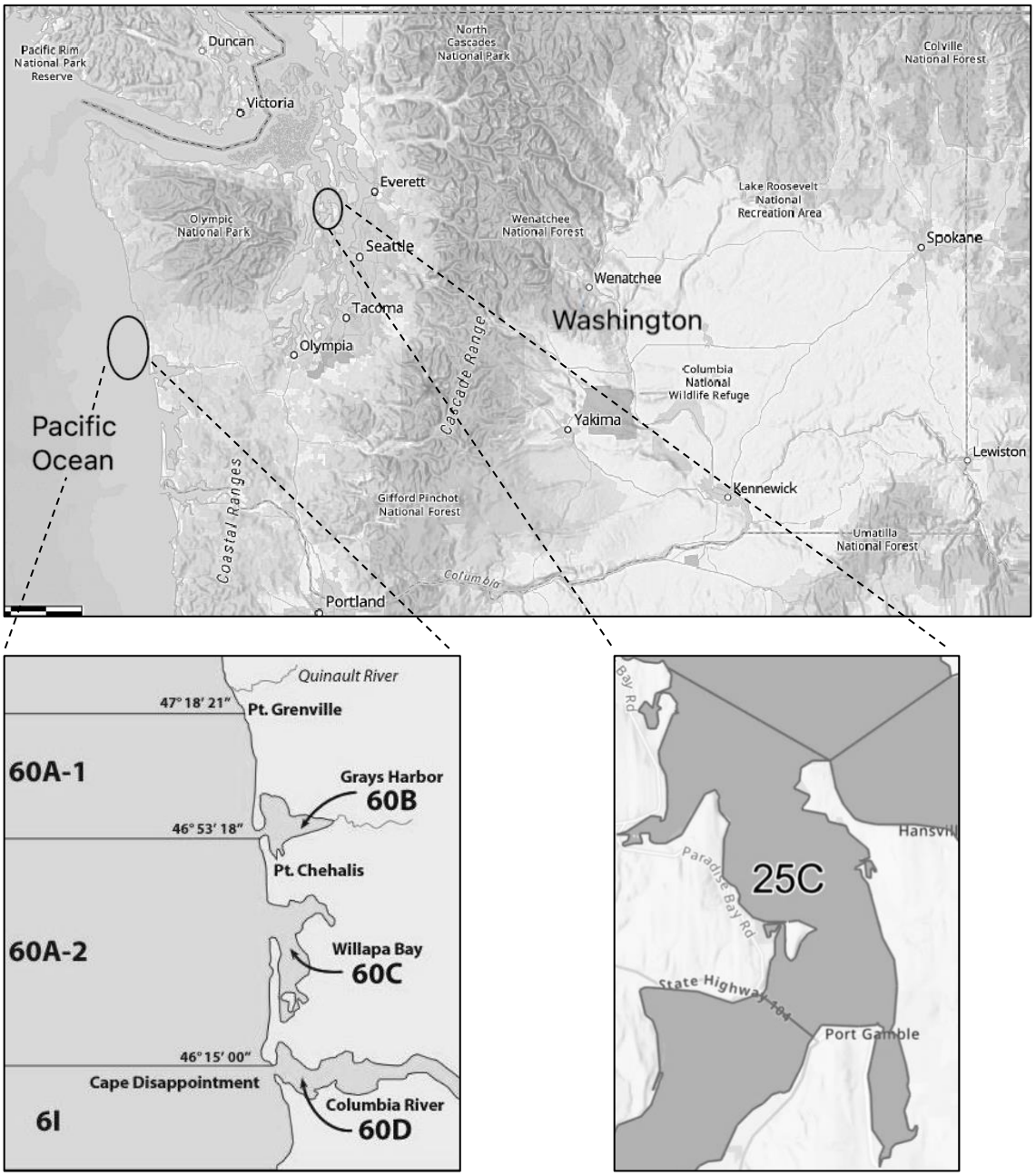
The Washington coast samples consisted of 20 Dungeness crabs that were donated from the Quinault tribe. These crabs were collected from catch area 60A-1 (Figure 3) at shallow depths between 0 – 300 meters, closer to the shore. The boundary that the crab were caught from extended from Point Grenville at 47^{o} 18' 21'' to the outermost exposed end of the south jetty and outside the mouths of tributary streams at 46^{o} 53' 18'' (Fuller 2023). Sampling treatment in this study followed standard operating procedure by West et al. (2012). This includes the sample

collection and handling, sample processing, tissue extraction, homogenization and composting, and preservation to limit possible contamination for contaminant analyses. Live Dungeness crabs were placed in coolers filled with ice and sea water to preserve the specimens and render them insensible as they were transported to the Evergreen State College labs. Upon arrival to the labs, crabs were ethically euthanized by rapidly destroying all the nerve systems through a procedure known as "spiking" (RSPCA). Crab samples were then rinsed with DI water and placed in individual gallon sized Ziplock bags and stored in the freezer at approximately -15ºC – 20ºC until sample processing occurred a week later.

Site selection and sampling effort was based on collecting Dungeness crab in two areas with different anthropogenic influences to track possible exposure patterns of toxic contamination, areas that typically are fished by sport and tribal fishers, and to support an evaluation of seafood safety for a human health risk assessment. Both sampling areas do share some anthropogenic influences that could potentially lead to heavy metal contamination such as vehicle and boat related phenomena, and stormwater runoff. However, the Hood Canal sampling location within the Puget Sound represents a more urbanized area with potentially more anthropogenic influences, whereas the Washington coast is more rural in comparison.

Figure 1.

Map of Washington State and Sampling Sites



Note. Dungeness crab sample site locations in the Washington coast (WDFW catch area 60A-1) and the Puget Sound (Hood Canal) (WDFW catch area 25C).
Sources: https://tribalgis.maps.arcgis.com;WSDOT;https://wdfw.wa.gov.

Tissue Dissection

When processing the specimens for contaminant analysis, anything (work surfaces, instruments, etc.) that would come in contact with the portion of the specimen being analyzed was cleaned beforehand in accordance with West et al. (2012) protocol. Dissection tools were pre-cleaned between resecting individual crab samples by hand washing in warm lab grade detergent water, thoroughly rinsed under tap water, followed by a DI water rinse three times. Lastly, they were solvent cleaned by spraying isopropyl alcohol on them and left to air dry on non-metal dissection boards to prevent cross contamination. Additionally, all sample jars were acid washed in 4 M nitric acid for four hours or more, followed by a DI water rinse three times before use.

Sample bags of whole crabs were removed from the freezer and allowed to thaw just enough for processing. Crab tissue samples were dissected to obtain the edible parts (muscle and hepatopancreas). Hepatopancreas samples were removed by separating the carapace from the body, scraping the organ tissue from the body cavity into a polypropene sample jar, homogenized using a tissue blender, weighed, and finally stored in the freezer. Forty grams of muscle tissue were resected from the claws and sections of the legs using pre-cleaned "seafood forks", scissors, and forceps, placing them in a polypropene sample jar, homogenized with a tissue blender, weighed, and finally placed in the freezer at approximately -15^{o}C – 20^{o}C until sample digestion. Additionally, two more subsamples (five grams each) were taken from the processed muscle tissues and the hepatopancreas for every five crabs resected, and then distributed to additional labeled jars for a total of three subsamples of the same hepatopancreas and muscle tissue, then finally placed in the freezer for several weeks until sample digestion. Hood Canal crabs generated a total of 21 muscle tissue samples and 21 hepatopancreas samples for heavy metal analysis. Within the Washington coast crabs, 28 muscle tissues samples and 28

hepatopancreas samples were generated for heavy metal analysis. Altogether, a total of 98 tissue samples were analyzed for possible arsenic and lead contamination.

Acid Digestion

Acid digestion of the soft tissue samples was performed using EPA method 200.3 to prepare for heavy metal analysis (EPA 1991). All glassware used for the analysis were acid washed in 4 M nitric acid (HNO_3) for four hours or more, followed by a DI water rinse three times to avoid possible contamination. Five grams of frozen tissue sample was placed in a digestion tube with 10 ml of nitric acid and warmed on a digestion block to near boiling until the solution turned brown. The sample was removed from the block and allowed to cool, then an additional 5 ml of nitric acid was added to the mixture and continued heating until the solution became brown again. Once again, the solution was removed and allowed to cool, then 2 ml of nitric acid was added and continued to be heated until the volume was reduced to 5 ml. The sample was then removed to cool, and 2 ml of hydrogen peroxide (30%) was added, then continued to heat until the volume again was reduced to 5 ml. This step was repeated until the solution was clear or a total of 10 ml of hydrogen peroxide had been used. The digested samples were then filtered through a Whatman no.5 filter paper and glass gravity funnel into a 250 ml polypropylene bottle, then diluted to 100 ml. The final dilution of the digested samples for instrument analysis included transferring 400 µl of each solution into individual falcon tubes (15 ml), then diluting with DI water up to a 10 ml fill line, and finally being placed in the refrigerator until instrument analysis. The remainder of the digestion solution not being analyzed was also returned to the refrigerator for storage. This process was required to have a recommended 0.2 % Total Dissolved Solids (TDS) under the instrumentation protocol to limit sample-specific matrix effects and the potential for nebulizer blockage of the ICP-MS interface that can lead to signal drift and frequent maintenance.

Instrument Analysis

Determination of arsenic concentrations in the samples were analyzed using the Inductively Coupled Mass Spectrometer (ICP-MS) (Perkin Elmer Elan DRC-E) (See Appendix C: Table 12). The ICP-MS is a qualitative multi-element measuring systems that offers a wide detection range of elements. The ICP-MS uses an argon plasma source to dissociate the sample into its basic atoms or ions (Helaluddin et al. 2016). The ions are released from the plasma and funneled into the mass spectrometer, where they are isolated according to their atomic mass-to-charge ratio by a quadrupole mass analyzer. Samples are introduced to the plasma torch in an aerosol form, therefore liquid samples were required to nebulize. The liquid sample is pumped from a falcon tube via the peristaltic pump, and the high number of ions produced combined with low background interference provides a good detection limit for most elements. In addition, the ICP-MS offers the ability to provide the speciation of multi-elements simultaneously (Ray et al. 2004) when coupled with chromatographic systems such as ion-exchange high-performance liquid chromatography (HPLC) or gas chromatography (Wilschefski et al. 2019); however, the ICP-MS instrument alone was used in this study, thus unable to differentiation inorganic vs organic speciation of multi-elements. Due to this, total arsenic is reported by the instrument. Because inorganic arsenic (iAs) is of primary interest, this was calculated and reported in this thesis by using 1% of the total value of arsenic (Francesconi et al. 1993).

The ICP-MS was calibrated using diluted high-purity grade purchased element external standards ranging from 0.9 parts per billion (ppb) up to 90 ppb (Table 3). To verify the accuracy of the analytical method and assess any background contamination originating from the sample processing and preparations, method blanks (DI water), rinse blanks, subtraction blanks, internal standard (yttrium), and a singular element Quality Controls (QC's) were used. QCs were used to verify the acceptable on-going instrument performance and calibration standards under EPA

protocol 200.8. The QCs prepared were within the acceptable ranges of ±10% of the stated QCs value. Descriptive statistics to access precision of the instrument, included calculating the coefficient of variation for replicate samples and QCs. To minimize matrix effects, all standards and blanks had a minimum of 1% nitric acid (HNO_3) in the solution to matrix match digested samples to calibration standards, blanks, and QCs. All metal concentrations found were expressed in wet weight (ww) basis.

Spectral interferences represent one of the greatest limitations hampering the ICPMS determination in elemental speciation. Elements in their inorganic speciation form, such as arsenic, suffer from serious spectroscopic overlap with some interferent ion. In particular, $^{75}AS^{+}$ can produce a polyatomic ion in the argon plasma formed from the matrix product if it contains chloride (Cl), which is present in crab tissues from seawater. To correct for this, an interference correction equation following the EPA Method 200.8 protocol was entered into the instrument software to automatically calculate the extent of the interference and subtract this contribution to yield the correct concentrations.

Table 5.

Working External Standards to Calibrate the ICP-MS

	Low	Mid 1	Mid 2	Mid 3	High
Arsenic	0.92 ppb	4.91 ppb	19.14 ppb	48.98 ppb	90.08 ppb

Note. Arsenic external working standards representing the range of expected analytes found in the crab tissues expressed in ppb.

Statistical analysis

The software program JMP was used for all statistical analyses. A Shapiro-Wilk test was conducted of all grouped samples to test for normal distribution. If the grouped samples were considered not normality distributed, then the data was transformed using the natural log (Ln) of the values, then retested for normality using a Shapiro-Wilk Test. If the grouped samples continued to fail the Shapiro-Wilks test for being normal distributed, then a non-parametric Mann Whitney U test or a Wilcox signed rank test was used. Statistical significances were based on a 95% confidence interval (α=0.05).Using JMP, a two sample T-test was used to assess whether there was a statistically significant between the mean concentration of iAs in the muscle tissue and hepatopancreas between sample sites. Additionally, a paired one-sided T-test was run at each sampling location to compare the hepatopancreas to the muscle tissue to determine if the hepatopancreas was significantly higher than the muscle tissue. This was based on previous studies reporting lower concentrations in other body parts of crab, such as muscle tissue in comparison to the hepatopancreas (Cogun et al. 2017). In addition, a correlation analysis was run at each sampling site to determine if there was a strong positive relationship between the hepatopancreases and muscle tissues iAs concentrations. Lastly, a two-sided T-test was performed to compare the mean analytical concentrations of iAs to a reference material to determine if they were above or below the certified values to be considered safe to consume.

Reference materials consisted of the healthy recommended Screening Value (SV) for a noncarcinogen from the EPA. The following equation was used to calculate SVs for noncarcinogens:

$$SV_n = (RfD \times BW)/CR$$

SV_n = Screening value for a noncarcinogen (mg/Kg; ppm)

RfD = Oral reference dose (mg/kg/d)

BW = Mean body weight of general population or subpopulation (kg)

CR = Consumption Rates of organism for selected subpopulations (kg/d)

The RfD is an estimate of a daily oral exposure to the human population (including sensitive groups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (EPA 2014). The EPA reference dose (RfD) for inorganic arsenic used was 0.3 µg/kg/day (0.0003 mg/kg/d)) (EPA 2012a), whereas kg refers to the body weight of the individual ingesting the Dungeness crab. Body weight (BW) values are the average body weights corresponding to various population groups (i.e., adult men and women, children, and adolescents) (Table 6) (EPA 2000). The BW value of 70 kg, corresponding to the average body weight of an adult, was used. Consumption rates (CR) are the mean daily consumption rates of the Dungeness crab by the general population or subpopulation of concern averaged over a 70-year lifetime (Table 7). The CR default value of 17.5 g/d (0.0175 kg/d), corresponding to general and recreational fisher, was used. In addition, other CRs, corresponding to subsistence fishers (142.4 g/d; 0.142 kg/d), average Native American subsistence fishermen (70 g/d; 0.07 kg/d), and Native American subsistence fishers estimated 95th percentile (170 g/d; 0.17 kg/d) were used. These additional CR values were used to represent a wide range of subpopulations that could be at greater risk of having higher body burden of bioaccumlative contaminants in comparison to the default CR. Lastly, the SV values were converted from mg/kg to µg/kg (ppb). All these components, combined to calculate the SVs, give us numerical estimates of the adult preventative risks of consuming these Dungeness crabs. As stated earlier, due to the limitations of the ICP-MS being

unable to identify the inorganic vs. organic speciation of arsenic present, assumptions were made based on previous literature that have found inorganic arsenic concentrations found in seafoods to range from >1% to 20% of the total arsenic concentration (Edmonds and Francesconi 1993). Thus, 1% of the total arsenic present was signified as iAs, and the rest of the arsenic present was determined to be organic.

Table 6.

Recommended Values for Mean Body Weight (BWs)

Variable	Recommended value	Subpopulation
BW	70 kg	All adults (U.S. EPA, 1999a)
	78 kg	Adult males (U.S. EPA, 1985b, 1990a)
	65 kg	Adult females (U.S. EPA, 1985b, 1990a)
	12 kg	Children <3 yr (U.S. EPA, 1985b, 1990a)
	17 kg	Children 3 to <6 yr (U.S. EPA, 1985b, 1990a)
	25 kg	Children 6 to <9 yr (U.S. EPA, 1985b, 1990a)
	36 kg	Children 9 to <12 yr (U.S. EPA, 1985b, 1990a)
	51 kg	Children 12 to <15 yr (U.S. EPA, 1985b, 1990a)
	61 kg	Children 15 to <18 yr (U.S. EPA, 1985b, 1990a)

Note. Body weight (BW) dose response variable used to calculate the Screen Values for inorganic arsenic suggested by EPA. Source: U.S. EPA 2000.

Table 7.

Fish Consumption Rates for Various Fisher Populations

Source	Recreational Fishers (g/d)	Subsistence Fishers (g/d)	Native American Subsistence Fishers (g/d)	Native Americans (g/d)	Basis for Consumption Rate
U.S. EPA	17.5 [a]	142.4 [a]	70 (mean) [b] 170 (95th percentile)[b]	NA	Fish consumption rate from 1994 and 1996 Continuing Survey of Food Intake by Individuals (CSFII)
Harris and Harper (1997)	NA	NA	540 (fresh, smoked and dried)	NA	Surveyed members of the Confederated Tribes of the Umatilla Indian Reservation
CRITFC (1994)	NA	NA	NA	59 (mean) 170 (95th percentile) 390 (99th percentile)	Surveyed members of the Umatilla, Nez Perce, Yakama, and Warm Springs Tribes
Toy et al. (1996)	NA	NA	NA	53 (median, males) 34 (median, females)	Surveyed members of the Tulalip Tribe
				66 (median, males) 25 (median, females)	Surveyed members of the Squaxin Island Tribe

Note. Consumption rates (CR) from various populations used to calculate the Screening Values for inorganic arsenic suggested by EPA. Source: U.S. EPA 2000

Results

The concentrations of inorganic arsenic in Dungeness crab tissues from the two sampling locations were determined by using the ICP-MS and identified to levels of potential concern to human health based on the frequency of detection, contaminants concentrations, and associated toxicity. The muscle tissue crab samples did not vary significantly in levels of iAs contamination between the Hood Canal natural log transformed mean (m= 3.43, SE = 0.11 µg/kg wet weight) and Washington coast (m=3.45, SE=0.09 µg/kg wet weight) ($t(47)=0.04$, $p > 0.05$; Figure 2). In contrast, hepatopancreas samples did vary significantly in levels of iAs contamination between the Hood Canal (m= 22.37, SE= 2.86 µg/kg wet weight) and Washington coast (m=31.39, SE= 2.47 µg/kg wet weight) ($t(33)=2.37$, $p < 0.05$; Figure 3); however, Washington coast crabs were higher in comparison to the Hood Canal. There was no significant difference between muscle and hepatopancreas iAs concentrations in the Washington coast samples (m= -1.78,SE=10.35 µg/kg wet weight) ($W= -63$, $p > 0.05$; Figure 5). In addition, the natural log transformed Hood Canal hepatopancreas and muscle tissue samples were not significantly different in levels of iAs (m= -16.72, SE= 3.89 µg/kg wet weight)($t=-4.28$, $p>0.05$; Figure 4).

The natural log transformed hepatopancreas and muscle tissue iAs concentrations were positively correlated for Hood Canal samples ($r =0.4313$) (Figure 4). Similarly, the natural log transformed hepatopancreas and muscle tissues iAs concentrations were positively correlated in Washington coast samples, with an even stronger positive correlation ($r =0.7766$) (Figure 5).

The natural log transformed mean concentrations of iAs from the Washington coast were not significantly higher than the EPA screening values (SV) of 1200 µg/kg/day, corresponding to recreational fishers (m=3.49, SE= 0.046 µg/kg wet weight) ($t(55)= -77.49$). Additionally, the

natural log transformed mean concentrations of iAs from the Hood Canal were not significantly higher than the EPA screening values (SV) of 1200 µg/kg/day, corresponding to recreational fishers (m=3.26, SE=0.094 µg/kg wet weight) ($t(41)= -40.34$, $p > 0.05$); therefore, inorganic arsenic concentrations for each sampling location were lower than the threshold that would indicate unhealthy consumption. In addition, comparison of mean concentrations of iAs from both sampling sites were below all the alternative EPA SVs corresponding to subsistence fishers, average Native American subsistence fishers, and the highest 95th percentile of Native American subsistence fishers.

In terms of the ICP-MS, the calibration ranges and linearity of the method are reported in Table 8. The accuracy of the method was assessed by comparing concentrations of the quality control (QC) in the range of the calibration curve with their true values. Results indicated the accuracy of all levels of QC were within the ±10% of the true values (see Appendix C: Table 13). The average QC samples deviated by 4.52 % of the theoretical value. The linear coefficient of determination (R^2) value for the response function was 0.9990, therefore indicating reasonable linearity and our ability to accurately predict the concentrations within the tissue samples using our method with little uncertainty. The accuracy of the instrument and method was analyzed using the coefficient of variation (CV) among replicate tissues samples with the mean CV being 3.76 % and ranged from 0.21 % to 14.57% (Appendix C: Table 14).Table 9 shows the concentrations of estimated inorganic arsenic (iAs) in the muscle tissue and hepatopancreas from each sampling location. In addition, the calculated screening value (SV) limit of inorganic arsenic using different consumption rates (CR) of subpopulations is at the bottom of the table.

Table 8.

Calibration Ranges and Linearity

Element	Concentration level (ppb)	Q Equation	R^2	Internal standard
^{75}As	1-90	Y=0.00713589x + 0.00011264	0.9990	Y

Note. The Equation of the trend line of best fit for the external standards utilized to calibrate the ICP-MS for As detection.

Table 9.

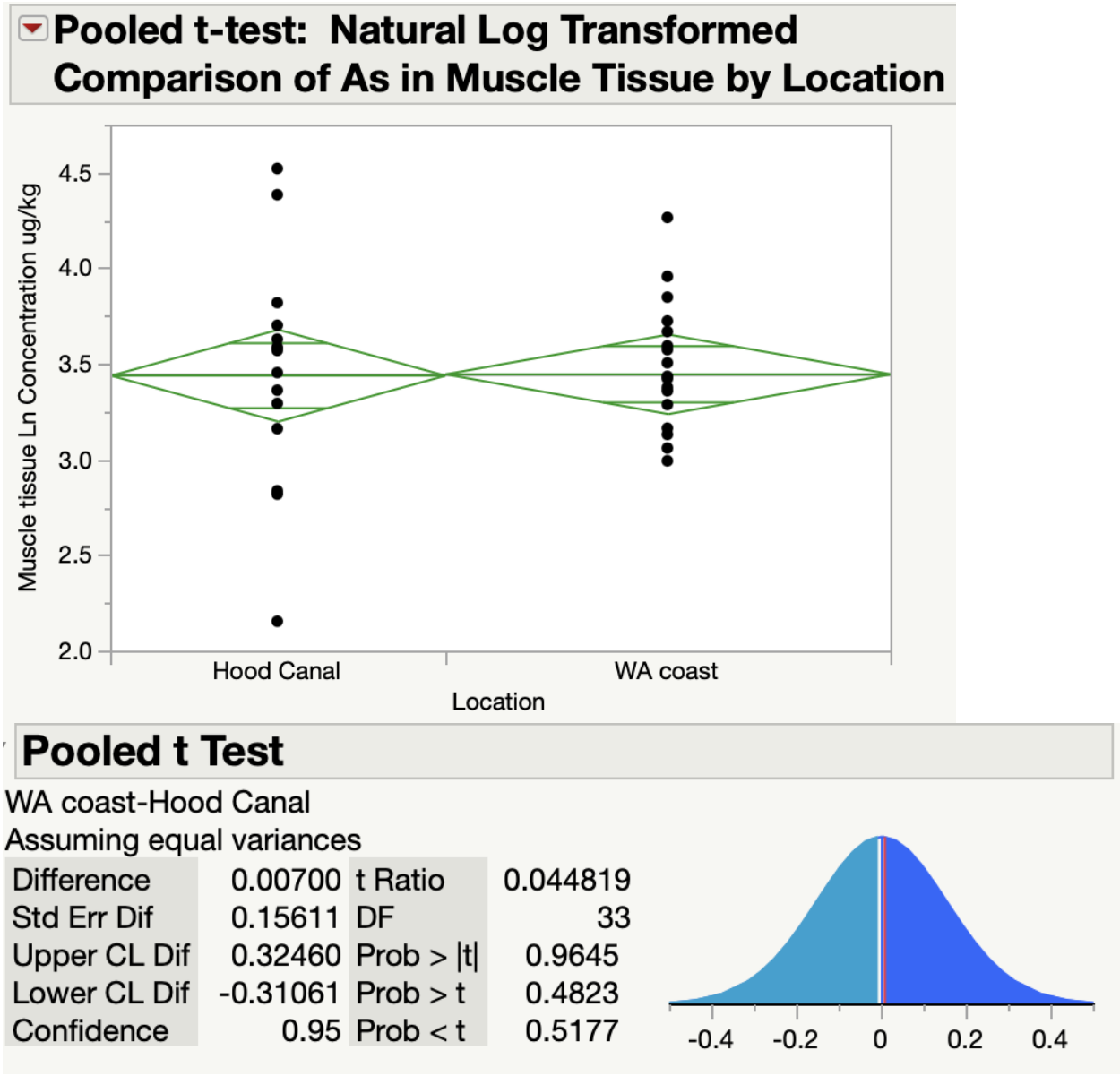
Concentrations of Arsenic Detected in Dungeness Crabs Within Sampling Sites.

Sample Site	Tissue Type	Analyte	Mean concentration (µg/kg)	Min/Max (µg/kg)	±SE (µg/kg)
WA Coast	Muscle	iAs	33.27	19.93 – 71.12	2.77
WA Coast	Hepatopancreas	iAs	31.39	15.52 – 64.14	2.65
Hood Canal	Muscle	iAs	36.63	8.61 – 91.88	5.78
Hood Canal	Hepatopancreas	iAs	22.37	7.17 – 46.95	2.61
Limit Levels in Crabs					
EPA	CR-Recreational fisher	iAs	1200 µg/kg/day		
	CR- Subsistence fisher	iAs	147 µg/kg/day		
	CR- Average Native American Subsistence fisher	iAs	300 µg/kg/day		
	CR- Native American Subsistence fisher (95^{th} percentile)	iAs	123 µg/kg/day		

Note. Mean, min/max, and standard error of estimated inorganic arsenic concentrations found within the Hood Canal and WA coast, and guideline limit levels for EPA. EPA limit level based on different consumptions rates of sub populations. Numbers expressed in µg/kg wet weight basis.

Figure 2.

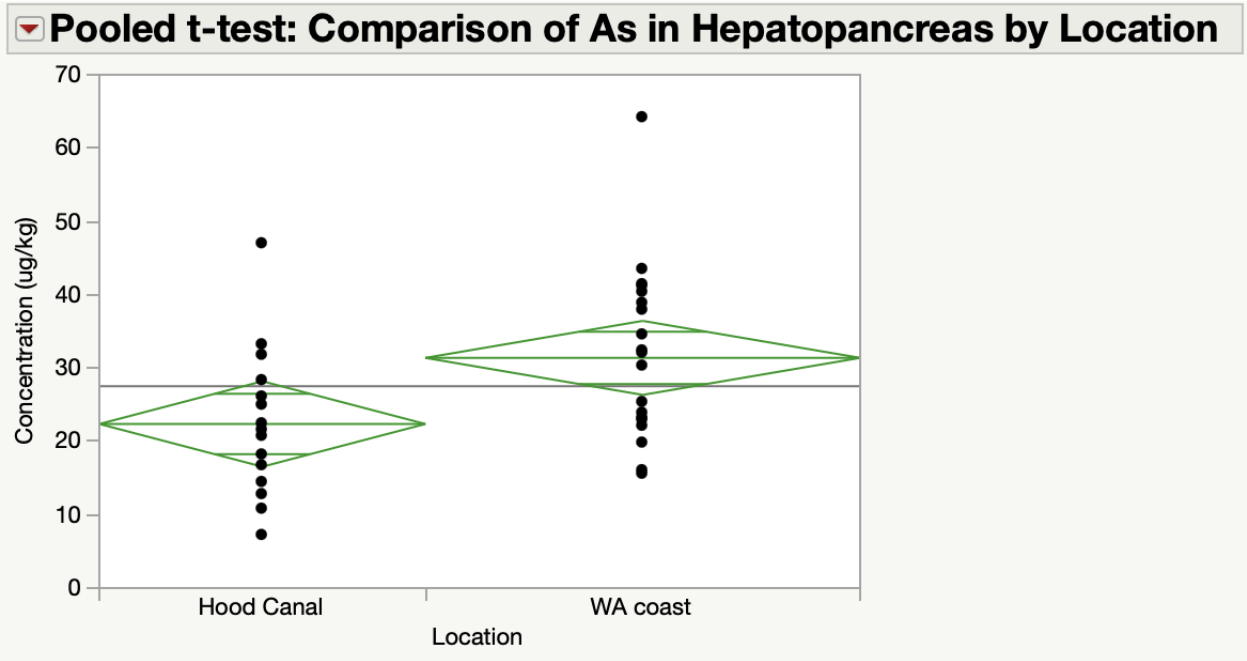
Comparison of Inorganic Arsenic in Muscle Tissue Between Sites



Note. Results of two sample t-test comparing mean muscle tissue concentrations of inorganic arsenic (ug/kg wet weight) between sampling sites.

Figure 3.

Comparison of Inorganic Arsenic in Hepatopancreas Between Sites

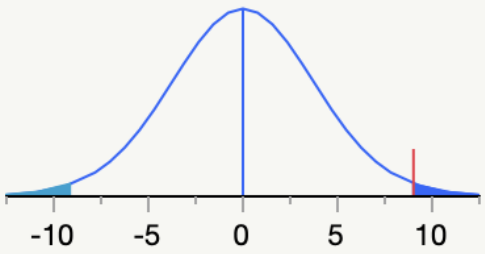


Pooled t Test

WA coast-Hood Canal

Assuming equal variances

Difference	9.0150	t Ratio	2.379991
Std Err Dif	3.7878	DF	33
Upper CL Dif	16.7214	Prob > |t|	0.0232*
Lower CL Dif	1.3086	Prob > t	0.0116*
Confidence	0.95	Prob < t	0.9884

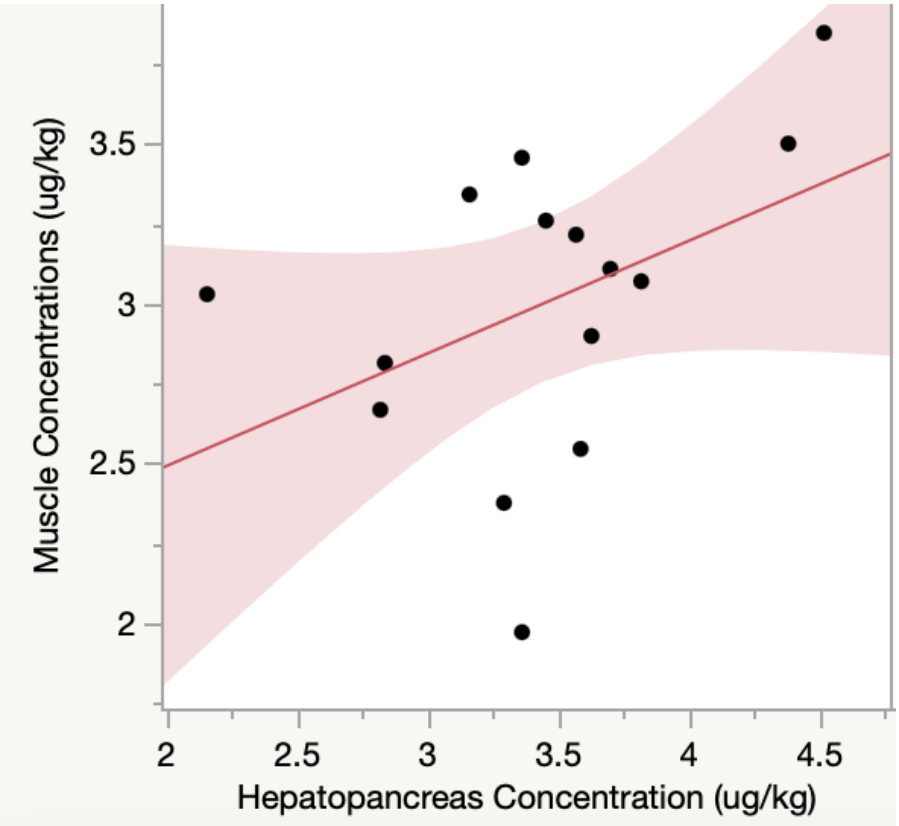


Note. Results of two sample t-test comparing mean hepatopancreas concentrations of inorganic arsenic (ug/kg wet weight) between sampling sites.

Figure 4.

Correlation Between Hood Canal Muscle Tissue and Hepatopancreas

	Ln (Muscle)
Ln (Hepatopancreas)	0.4313

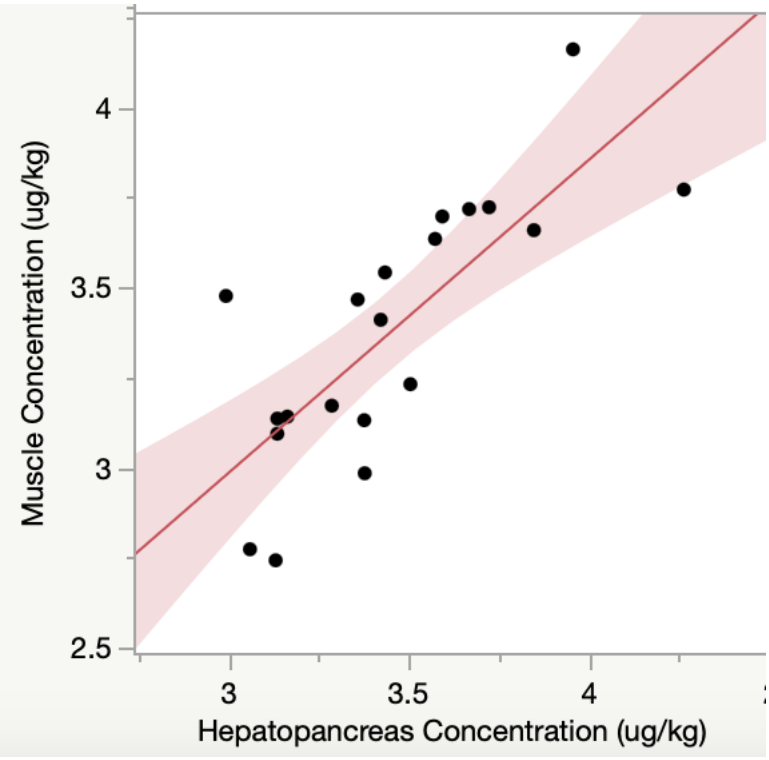


Note. Hood Canal correlation analysis between muscle tissue and hepatopancreas concentrations (ug/kg wet weight).

Figure 5.

Correlation between Washington Coast Muscle and Hepatopancreas

	Ln (Muscle)
Ln (Hepatopancreas)	0.7766



Note. Washington coast correlation analysis between muscle tissue and hepatopancreas concentrations (ug/kg wet weight).

Discussion

The primary objective of this thesis was to investigate the levels of inorganic arsenic accumulation in Dungeness crabs caught within the Puget Sound (Hood Canal) and on the Washington coast as well as determine if accumulation levels present a human health risk in the consumption of these species. In addition, to our knowledge, this study was the first assessment of examining inorganic arsenic contamination along the Washington outer coast, as well as the first comparison of inorganic arsenic contamination between the Puget Sound (Hood Canal) vs. the Washington coast. Particular concern with this study is the identification of potential exposure of heavy metals to Washington residents in the consumption of Dungeness crabs. In addition, the possible ramifications of identifying chronic accumulation in a marine organism that provides key economic, recreational, and cultural significance for tribal and non-tribal populations in WA. Arsenic occurs naturally in seawater, so we expected to observe some presence in all tissue samples. However, analysis revealed notable findings with ubiquitously low accumulation of inorganic arsenic at the two sample sites and in the two Dungeness crab tissue types. These results signify that Dungeness crabs have the ability to accumulate iAs in its body tissues as well as could correspond to iAs presence in the benthic sediment or water column in the Hood Canal and on the Washington Coast. In the Hood Canal, our estimated iAs concentrations means ranging from 23.25 ug/kg to 39.97 ug/kg were higher in comparison to Johnson et al. (2002), who found inorganic arsenic levels to be 2.6 ug/kg. In addition, a few outlier crab samples contained iAs concentrations ranging from 80 ug/kg to 93 ug/kg. Despite the few outliers, the mean concentrations found could still be comparable to reference areas with little contamination in the Puget Sound, ranging from 0.47 ug/kg to 44 ug/kg (Johnson et al. 2002; Johnson et al. 2000), thus appearing near background levels of typical urbanized waters.

In general, the presence of iAs warrants the necessity of analyzing possible human health risks, however, through our health risk assessment, all concentrations were under the EPA recommended Screening Values (SV) for all different scenarios of consumption rates, thus could be deemed safe for human consumption.

Another heavy metal, lead, was attempted to be analyzed in the crab tissues sample using the ICP-MS; however, no consistent patterns were observed in the external standards, blanks, QCs, and crab samples. Therefore, the results were deemed inaccurate and omitted from the study. Several common issues could have occurred leading to the lack of precision, such as carryover, drift, and degraded detection limits. Although the percentage of relative standard deviation (RSD) was lower (less than 5) the lower the RSD, the better precision, the ICP-MS continued to have problems with precision and accuracy, which could have been a result of problems in the sample introduction system. Additionally, although we used the recommended <0.2 % (2g/L) total dissolved solids (TDS), there was potential for nebulizer blockage (Wilschefski et al. 2019), which could have led to poor estimation of lead concentrations within the samples.

Although the methodology used under the EPA reference guides would deem the Dungeness crab samples safe for human consumption, the methodologies does involve levels of uncertainties. For example, in calculating the SVs for inorganic arsenic, the use of the RfD value, which is the estimate of a daily oral exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime (EPA 2014), may vary by up to an order of magnitude. Additionally, the RfD only partially incorporates factors such as individual characteristics including underlying health status and medications, baseline dietary consumption and quality, genetics, socioeconomic status, age, gender, and pregnancy that can

result in increased sensitivity to a chemical pollutant (Harper et al. 1999). Furthermore, the SVs alone are used to provide protective exposure limit of noncarcinogenic effects rather than to predict risk with levels of uncertainty intended to account for the variation in sensitivity among members of the human population (EPA 2014). Therefore, if the values found in this present study were above the SVs, it wouldn't tell you the risk involved with continued chronic exposure over a lifetime, but just that values above the limit could cause adverse reactions that have been previously observed.

In using different consumption rate scenarios in this study, we tried to establish sufficient real-world scenarios that incorporate variations of sub-populations of interest. However, in our scenario of using only adults body weight, we left out a recognized sensitive group, children. Additionally, the EPAs recommended consumption and exposure rate of 170 g/d for the 95th percentile of Native American subsistence fishers could be an underestimate in comparison to various tribes that consume Dungeness crabs from either sampling area. As previously forementioned, identifying and incorporating local fish and shellfish consumptions rate for nontribal or tribal populations is pertinent in established proficient health standards for local populations. For all of these reasons, it should be noted that risk assessments through the EPA methodologies are partial guides to establish seafood consumption guidance or advisories. Lastly, due to the lack of data on the chemical form of arsenic present, we relied on assumptions in accordance with previous literature's results by using 1% of the total arsenic determined, thus lacking in the exact estimations of harmful inorganic arsenic. Therefore, the significance of our findings is uncertain and impossible to make precise taxological discussions. However, we simulated extreme scenarios, in which the assumption of 20% of the total arsenic found was inorganic and values were still significantly lower than the EPA SVs in both sampling sites using

the consumption rate default value, corresponding to general and recreational fishermen. Thus, the concentrations found in this study should not present any human health risk. Overall, continued further sampling and analysis for arsenic using advanced analytical techniques to differentiate total arsenic and inorganic arsenic species could strengthen our evidence of accumulation patterns found within this study and its relevance as a risk to human health.

During the analysis of total arsenic present using the ICP-MS, samples were mixed with the internal standard Yttrium in order to compensate for variability in signal intensity due to ion suppression caused by the matrix components and minimize the effects of random and systematic errors during the analysis (Bradshaw 2023). During this process, variations in internal standard recoveries are normal. However, during our analysis, the internal standard variation was 7.8% between all samples. Thus, there are possible suspicions in the amount of internal standard not being added correctly to the solution, either due to pipetting or analyst error. In addition, excessive recovery in the samples could be an indication the internal standard was in the original samples, with Yttrium being found in marine ecosystems (Andrade et al. 2023). Based on these variations in the internal standard recoveries, the calculated concentrations of total arsenic could be variable from what we determined, leaving levels of uncertainty if the study were repeated; however, we speculate that the accumulation patterns observed would likely remain consistent. In addition, our estimated inorganic arsenic concentrations being four times below the lowest EPA's SVs leaves availability for levels of uncertainty. However, replication of the study in the future would verify our assertions.

With analyzing both the hepatopancreas and muscle tissue of Dungeness crabs, we hoped to strengthen our understanding of accumulation patterns, particularly in terms of comparing accumulation within different edible tissues. The Hepatopancreas is a vital organ in crustaceans

that plays a significant role of absorbing and storage of nutrients, as well as detoxifying foreign substances such as heavy metals (Wang et al.2014), therefore having a high affinity to bind with heavy metals. Conversely, previous studies have reported lower concentrations in other body parts of crab, such as muscle tissue, thus baring lower risk of potential exposure to these harmful pollutants (Cogun et al. 2017). In this present study, this accumulation pattern was not observed with arsenic not being significantly higher in the hepatopancreas vs the muscle tissue in comparing the difference within each sampling site, therefore being the opposite of our hypothesis. The reasoning behind this could be implicated by the Dungeness crab's ability to metabolically regulate these metals, resulting in low accumulation found in the hepatopancreas, however overall, we have no clear explanation for the discrepancy in this accumulation pattern. In addition, the correlation analysis revealed a strong positive relationship between the Washington coast hepatopancreas and muscle tissue iAs concentrations. However, the correlation analysis revealed only a moderate positive relationship between the muscle tissue and hepatopancreas iAs concentrations in the Hood Canal, therefore implicating that there is variability in accumulation patterns among the organisms. Furthermore, the hepatopancreas' role in the storage of nutrients (heavy metals) that can later be transported throughout the muscle tissues could explain the positive correlation observed in this study. Taken as a whole, this study adds to the collection of knowledge in understanding the role in which this crustacean may accumulate pollutants in different tissue types.

Concentrations of inorganic arsenic did not appear to show a distinct relationship between accumulation patterns and increased anthropogenic activities by the lack of significant difference in the iAs concentrations in the muscle tissues from the Hood Canal vs Washington coast. In contrast, hepatopancreas samples were significantly higher in the Washington coast vs

the Hood Canal, therefore finding mixed results. This could suggest that either these two marine ecosystems could share more anthropogenic activates than we previously thought or that accumulation patterns are possibly unrelated to proximity to human activities. In general, proximity to anthropogenic activities such as boat marinas, industry, and housing developments provide sources of heavy metal input in nearby marine ecosystems (Akter et al.2005; Acosta et al.2010). Additionally, vehicle related phenomenon can result in fine grain sized road deposited sediments which can contain higher metal concentrations and can be quickly washed off through heavy rain events and discharged into nearby marine ecosystems (Jeong et al. 2020). Overall, though, this study did indicate the presence of inorganic arsenic accumulation; however, as previously forementioned, despite a few outlier crab samples having iAs concentrations ranging from 80 ug/kg to 93 ug/kg, iAs concentrations were relatively low, with means ranging from 22.37 ug/kg to 36.63 ug/kg and comparable to background levels of urbanized waters within the Puget Sound ranging from 0.47 ug/kg to 44 ug/kg (Johnson et al. 2002). Furthermore, these dynamic marine ecosystems make it difficult to confirm the primary sources of pollution and whether differences could be due to road-sourced metal contamination or other factors. Therefore, it cannot make concrete observations about identifying the causes of the patterns observed. Spatial analysis of watershed dynamics would be an impactful addition to this study to make for a more accurate and precision conclusion about considering the processes which influence sources of contamination. More research is required to understand the runoff pathways and circulation patterns in the Washington region to produce a better assessment of metal contamination sources.

Aside from anthropogenic activities suggested role in alterable accumulation patterns in Dungeness crabs, other factors could indicate this distribution process in the surrounding

environment of Washington Coast and Hood Canal. For instance, bioavailability of metals has been found to depend on processes such as chemical speciation, and influenced by environmental parameters such as salinity, temperature, and pH (Zhang et al. 2022) as well as impacting cellular processes in marine organisms. The Hood Canal, for example, historically produces poor water circulation that results in low dissolved oxygen (DO) (Newton et al. 2007), which has been identified to increase the levels of heavy metals in sediment (Surbakti et al. 2021). pH levels in Hood Canal have also been recorded to be as low as 7.4 (Feely et al. 2010). Low pH increases the solubility of heavy metals and can cause metal desorption from the sediments and organic ligands, resulting in a higher influx of dissolved metals into the water column (Ivanina et al. 2015), thus increasing the probability of accumulation in these crustaceans. The combination of low pH and low DO in Hood Canal could be used as an explanation for why Hood Canal would have higher concentrations of iAs in reference to the Washington coast, however this wasn't the case in our study, thus leaving unanswered questions. Though these environmental parameters were not collected in this present study, future studies should incorporate complementary data such as water and sediment quality alongside biological organisms, as well as seasonal pollution biomonitoring programs, to better conceptualize the bioaccumulative processes in the marine environment and their effects on human health.

Conclusion

Results of this study show that the Dungeness crabs caught within the Puget Sound (Hood Canal) and Washington Coast have low levels of inorganic arsenic contamination. In addition, despite a few outliers, in some instances, the concentrations detected could appear to be at background levels for Puget Sound. The hepatopancreas of the Dungeness crab were not consistent in having higher inorganic arsenic accumulation patterns relative to the muscle tissue in the two sampling areas. In comparing the two sampling areas, the Hood Canal did not have significantly higher levels versus the Washington Coast in both tissue types. The influence of urban/industrial sources on inorganic arsenic contamination in these marine ecosystems is still uncertain. In addition, to our best knowledge, this study was the first to examine inorganic arsenic patterns along the Washington outer coast. Inorganic arsenic concentrations found in both sampling sites under the assumptions of the study should not pose a significant human threat for tribal or recreational consumption of these Dungeness crabs caught within these areas. However, the significance of inorganic arsenic concentrations found is still uncertain. These results are important to strengthen awareness among people throughout Washington state of the possible risks associated with recurrent consumption of possible contaminated crabs, as well as the further necessity of continuing to characterize heavy metal contamination within the region.

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Appendix A- Acronyms and Abbreviations

Following are acronyms and abbreviations used frequently in this report.

As	Arsenic
Pb	Lead
iAs	Inorganic arsenic
AsB	Arsenobetaine
HM	Heavy metal
MMA	monomethylarsonic
DMA	Dimethylarsinic
DOM	Dissolved organic matter
HNO_3	Nitric acid
EPA	U.S Environmental Protection Agency
WHO	World Health Organization
QIN	Quinault Indian Nation
WA	Washington state
SPM	Suspended particular matter
DI	Deionized
DO	Dissolved oxygen
QC	Quality control
RSD	Relative Standard Deviation
SOP	Standard operating procedure
WDFW	Washington Department of Fish and Wildlife
ICP-MS	Inductively coupled mass spectrometer
TDS	Total dissolved solids
BW	Body weight
CR	Consumption rates
SV	Screening value

Units of Measurement

^oC	degrees Celsius
ww	wet weight
g	grams, unit of mass
Kg	kilograms, unit of mass equal to 1,000 grams
Mg	milligram, unit of mass equal to 0.001 grams
ug	microgram, unit of mass equal to 0.000001 grams
ppt	parts per trillion
ppm	part per million
ppb	parts per billion
Mg/kg	milligrams per kilogram (parts per million)
µg/g	micrograms per gram (parts per million)
µg/kg	micrograms per kilogram (parts per billion)
RfD	Reference dose (ug/kg-body weight/day)
SV	Screening value (mg/Kg; ppm)

Appendices B- Arsenic Raw Data For All Samples Analyzed

Table 10.

Arsenic Concentrations in Dungeness Crab Tissue From Washington Coast

Location	Sample ID	Tissue type	Analyte	Total Arsenic (µg/kg)	Estimated Inorganic Arsenic (µg/kg)
WA coast	1.M	muscle	As	3,064	30.63
WA coast	2.M	muscle	As	2,288	22.88
WA coast	3.M	muscle	As	2,675	26.74
WA coast	4.M	muscle	As	2,364	23.63
WA coast	5.M	muscle	As	7,388	73.87
WA coast	5.M rep 1	muscle	As	7,324	73.24
WA coast	5.M rep 2	muscle	As	6,626	66.26
WA coast	6.M	muscle	As	2,927	29.26
WA coast	7.M	muscle	As	3,327	33.27
WA coast	8.M	muscle	As	3,636	36.35
WA coast	9.M	muscle	As	5,227	52.27
WA coast	10.M	muscle	As	4,709	47.09
WA coast	10.M rep 1	muscle	As	4,642	46.41
WA coast	10.M rep 2	muscle	As	4,717	47.16
WA coast	11.M	muscle	As	4,140	41.40
WA coast	12.M	muscle	As	3,101	31.00
WA coast	13.M	muscle	As	1,993	19.93
WA coast	14.M	muscle	As	2,299	22.99
WA coast	15.M	muscle	As	2,913	29.12
WA coast	15.M rep 1	muscle	As	2,933	29.33
WA coast	15.M rep 2	muscle	As	2,776	27.75
WA coast	16.M	muscle	As	3,917	39.16
WA coast	17.M	muscle	As	2,299	22.98
WA coast	18.M	muscle	As	2,931	29.30
WA coast	19.M	muscle	As	2,131	21.30
WA coast	20.M	muscle	As	3,513	35.12
WA coast	20.M rep 1	muscle	As	3,569	35.68
WA coast	20.M rep 2	muscle	As	3,609	36.09
WA coast	1.H	Hepatopancreas	As	3,027	30.27
WA coast	2.H	Hepatopancreas	As	1,552	15.52
WA coast	3.H	Hepatopancreas	As	2,385	23.84
WA coast	4.H	Hepatopancreas	As	2,313	23.13

Table 10. (Continued)

Arsenic Concentrations in Dungeness Crab Tissue From Washington coast

Location	Sample ID	Tissue type	Analyte	Total Arsenic (µg/kg)	Estimated Inorganic Arsenic (µg/kg)
WA coast	5.H	Hepatopancreas	As	4,230	42.29
WA coast	5.H rep 1	Hepatopancreas	As	4,339	43.39
WA coast	5.H rep 2	Hepatopancreas	As	4,467	44.66
WA coast	6.H	Hepatopancreas	As	2,290	22.90
WA coast	7.H	Hepatopancreas	As	2,531	25.31
WA coast	8.H	Hepatopancreas	As	4,033	40.33
WA coast	9.H	Hepatopancreas	As	6,415	64.14
WA coast	10.H	Hepatopancreas	As	3,851	38.50
WA coast	10.H rep 1	Hepatopancreas	As	3,959	39.59
WA coast	10.H rep 2	Hepatopancreas	As	3,838	38.37
WA coast	11.H	Hepatopancreas	As	4,138	41.37
WA coast	12.H	Hepatopancreas	As	3,452	34.52
WA coast	13.H	Hepatopancreas	As	3,234	32.34
WA coast	14.H	Hepatopancreas	As	2,301	23.00
WA coast	15.H	Hepatopancreas	As	2,842	28.41
WA coast	15.H rep 1	Hepatopancreas	As	3,486	34.85
WA coast	15.H rep 2	Hepatopancreas	As	3,280	32.79
WA coast	16.H	Hepatopancreas	As	4,116	41.16
WA coast	17.H	Hepatopancreas	As	2,206	22.06
WA coast	18.H	Hepatopancreas	As	1,977	19.76
WA coast	19.H	Hepatopancreas	As	1,601	16.00
WA coast	20.H	Hepatopancreas	As	3,673	36.73
WA coast	20.H rep 1	Hepatopancreas	As	3,796	37.95
WA coast	20.H rep 2	Hepatopancreas	As	3,901	39.00

Note. Total arsenic and estimated inorganic arsenic concentrations (µg/kg) wet weight in Dungeness crab hepatopancreas and muscle tissue from crab collected in Washington coast. Sample ID: H = hepatopancreas, M= muscle tissue, rep = replication.

Table 11.

Arsenic Concentrations in Dungeness Crab Tissue From Hood Canal

Location	Sample ID	Tissue type	Analyte	Total Arsenic (ug/kg)	Estimated Inorganic Arsenic (ug/kg)
Hood Canal	1.M	muscle	As	862	8.61
Hood Canal	2.M	muscle	As	2,692	26.91
Hood Canal	3.M	muscle	As	2,885	28.84
Hood Canal	4.M	muscle	As	2,883	28.83
Hood Canal	5.M	muscle	As	1,701	17.00
Hood Canal	5.M rep 1	muscle	As	1,707	17.07
Hood Canal	5.M rep 2	muscle	As	1,706	17.06
Hood Canal	6.M	muscle	As	2,358	23.58
Hood Canal	7.M	muscle	As	8,012	80.11
Hood Canal	8.M	muscle	As	4,556	45.56
Hood Canal	9.M	muscle	As	3,549	35.49
Hood Canal	10.M	muscle	As	9,378	93.77
Hood Canal	10.M rep 1	muscle	As	9,041	90.41
Hood Canal	10.M rep 2	muscle	As	9,148	91.47
Hood Canal	11.M	muscle	As	1,675	16.74
Hood Canal	12.M	muscle	As	3,161	31.61
Hood Canal	13.M	muscle	As	4,048	40.48
Hood Canal	14.M	muscle	As	3,764	37.64
Hood Canal	15.M	muscle	As	3,663	36.63
Hood Canal	15.M rep 1	muscle	As	3,652	36.52
Hood Canal	15.M rep 2	muscle	As	3,518	35.18
Hood Canal	1.H	Hepatopancreas	As	2,069	20.69
Hood Canal	2.H	Hepatopancreas	As	1,076	10.75
Hood Canal	3.H	Hepatopancreas	As	717	7.17
Hood Canal	4.H	Hepatopancreas	As	3,174	31.74
Hood Canal	5.H	Hepatopancreas	As	1,529	15.29
Hood Canal	5.H rep 1	Hepatopancreas	As	1,526	15.26
Hood Canal	5.H rep 2	Hepatopancreas	As	1,949	19.48
Hood Canal	6.H	Hepatopancreas	As	2,829	28.28
Hood Canal	7.H	Hepatopancreas	As	3,318	33.17
Hood Canal	8.H	Hepatopancreas	As	2,155	21.54

Table 11. (Continued)

Arsenic Concentrations in Dungeness Crab Tissue From Hood Canal

Location	Sample ID	Tissue type	Analyte	Total Arsenic (ug/kg)	Estimated Inorganic Arsenic (ug/kg)
Hood Canal	9.H	Hepatopancreas	As	2,494	24.93
Hood Canal	10.H	Hepatopancreas	As	4,730	47.29
Hood Canal	10.H rep 1	Hepatopancreas	As	4,731	47.30
Hood Canal	10.H rep 2	Hepatopancreas	As	4,625	46.25
Hood Canal	11.H	Hepatopancreas	As	1,440	14.40
Hood Canal	12.H	Hepatopancreas	As	2,606	26.06
Hood Canal	13.H	Hepatopancreas	As	2,240	22.40
Hood Canal	14.H	Hepatopancreas	As	1,815	18.15
Hood Canal	15.H	Hepatopancreas	As	1,317	13.16
Hood Canal	15.H rep 1	Hepatopancreas	As	1,280	12.80
Hood Canal	15.H rep 2	Hepatopancreas	As	1,226	12.25

Note. Total arsenic and estimated inorganic arsenic concentrations (µg/kg) wet weight in Dungeness crab hepatopancreas and muscle tissue from crab collected in Hood Canal. Sample ID: H = hepatopancreas, M= muscle tissue, rep = replication.

Appendix C- Instrument Parameters and Assessment of the Accuracy

Table 12.

Instrument Parameters for the ICP-MS

Number of Replicates:	3
Peak Processing Mode:	Average
Signal Profile Processing Mode:	Average
Dual Detector Mode:	Dual
Dead Time (ns):	55
Power/W	1300
Plasma gas flow/L min^{-1}	15.0
Auxilary gas flow/L min^{-1}	0.5
Sample flow rate/mL min^{-1}	0.8

Note. Instrument Parameters for the ICP-MS used throughout the analyses.

Table 13.

Quality Control Accuracy

Analyte	Unit	Measured value	Target value	Deviation
As	Ug/L	4.95	4.70	5.12 %
As	Ug/L	5.03	4.70	6.48 %
As	Ug/L	5.06	4.70	7.16 %
As	Ug/L	4.91	4.70	4.29 %
As	Ug/L	4.98	4.70	5.66 %
As	Ug/L	4.82	4.70	2.59 %
As	Ug/L	4.65	4.70	1.14 %
As	Ug/L	4.93	4.70	4.72 %
As	Ug/L	4.97	4.70	5.43 %
As	Ug/L	4.52	4.70	3.96 %
As	Ug/L	4.92	4.70	4.51 %
As	Ug/L	4.82	4.70	2.57 %
As	Ug/L	4.96	4.70	5.16 %

Note. Quality control samples measured values vs target values to determine accuracy.

Table 14.

Accuracy Data for Replicate Measurements

Location	Sample ID	Arsenic concentration	CV
Hood Canal	5.M	3.40	0.21 %
Hood Canal	5.M rep 1	3.41	
Hood Canal	5.M rep 2	3.41	
Hood Canal	5.H	3.05	14.57 %
Hood Canal	5.H rep 1	3.05	
Hood Canal	5.H rep 2	3.89	
Hood Canal	10.M	18.75	1.87 %
Hood Canal	10.M rep 1	18.08	
Hood Canal	10.M rep 2	18.29	
Hood Canal	10.H	9.45	1.29 %
Hood Canal	10.H rep 1	9.46	
Hood Canal	10.H rep 2	9.25	
Hood Canal	15.M	7.32	2.24 %
Hood Canal	15.M rep 1	7.30	
Hood Canal	15.M rep 2	7.03	
Hood Canal	15.H	2.63	3.59 %
Hood Canal	15.H rep 1	2.56	
Hood Canal	15.H rep 2	2.45	
WA Coast	5.M	14.77	5.94 %
WA Coast	5.M rep 1	14.64	
WA Coast	5.M rep 2	13.25	
WA Coast	5.H	8.45	2.73 %
WA Coast	5.H rep 1	8.67	
WA Coast	5.H rep 2	8.93	
WA Coast	10.M	9.41	0.88 %
WA Coast	10.M rep 1	9.28	
WA Coast	10.M rep 2	9.43	
WA Coast	10.H	7.70	1.72 %
WA Coast	10.H rep 1	7.91	
WA Coast	10.H rep 2	7.67	
WA Coast	15.M	5.82	2.98 %
WA Coast	15.M rep 1	5.86	
WA Coast	15.M rep 2	5.55	

Table 14. (Continued)

Accuracy Data for Replicate Measurements

Location	Sample ID	Arsenic concentration	CV
WA Coast	15.H	5.68	10.26%
WA Coast	15.H rep 1	6.97	
WA Coast	15.H rep 2	6.55	
WA Coast	20.M	7.02	1.36%
WA Coast	20.M rep 1	7.13	
WA Coast	20.M rep 2	7.21	
WA Coast	20.H	7.34	3.00%
WA Coast	20.H rep 1	7.59	
WA Coast	20.H rep 2	7.80	

Note. Estimated inorganic arsenic concentrations (µg/kg) wet weight in Dungeness crab hepatopancreas and muscle tissue and calculated replicate coefficient of variation (CV) percentage. Sample ID: H = hepatopancreas, M= muscle tissue, rep = replication.