Characterization of present biological conditions in the intertidal community across Howe Sound, British Columbia

by

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Abstract

Howe Sound is a Pacific Northwest fjord located north of Vancouver, British Columbia. This fjord has been impacted by effluents from several industries including two pulp mills and a copper mine. After Environment Canada undertook more stringent enforcement on environmental standards in the 1980s, the Britannia copper mine and the Woodfibre pulp and paper mill were shut down. Historical data from the intertidal community indicate that recovery of the ecosystems in these areas has been minimal, particularly at sites in close proximity to industrial activities. The goal of this study was to begin a characterization of several present biological conditions in the intertidal community across Howe Sound. Six sites were selected and grouped based on degree of exposure to industrial activities. High exposure sites included Britannia Beach, Darrell Bay and Port Mellon, while moderate exposure sites included Porteau Cove and Lions Bay. Chaster bay was selected as a reference site for this study. Two biomarkers of exposure; ethoxyresorufin-O-deethylase (EROD) and metallothionein (MT) were measured in mussels to assess the availability of polyaromatic hydrocarbons and metals at these sites. When compared to the Chaster Bay reference site, EROD activity was significantly higher in mussels collected from the two high exposure sites (Britannia Beach (~2.1 increase) and Port Mellon (~1.5 increase)) and the two moderate exposure sites (Porteau Cove (~1.8 increase) and Lions Bay (~1.6 increase). MT levels were significantly higher in mussels from the Britannia Beach (~4.2 increase), Darrell Bay (~2.8 increase) and Porteau Cove (~2.4 increase). Results from another ecological bioindicator (giant kelp) showed that germination rates were significantly lower at Lions Bay, Port Mellon and Darrel Bay (> 60% reduction compared to the reference site). A 16% reduction in germination rate was also noted for Britannia Beach. Germination tube length were only found to be significantly reduced at Lions Bay (~20 % decrease), Darrell Bay (~37% decrease) and Britannia Beach (~10% decrease). Finally, in an intertidal community assessment using species richness as an index, Chaster Bay contributed 39.94% to the total species richness, a range of 17.78% - 19.02% for moderate exposure sites and 4.50% - 12.79% for the high exposure sites.

Keywords: biomarkers; bioindicators; Howe Sound; ethoxyresorufin-O-deethylase; pulp and paper mill; metallothionein

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List of Acronyms

AHR	Aryl hydrocarbon receptor
AMD	Acid mine drainage
AOX	Adsorbable organic halogens
ARNT	Aryl hydrocarbon nuclease translocation
BOD	Biochemical oxygen demand
CTMP	Chemi-thermo mechanical pulping
EROD	Ethoxyresorufin-O-deethylase
LCFA	Long chain fatty acid
LMW	Low molecular weight
MT	Metallothionein
PPM	Pulp and paper mill
TCDD	Tetrachlorordibenzo-p-dioxin
TFC	Total free chlorine
VOC	Volatile organic compounds

Chapter 1.

Introduction

Howe Sound is a fjord located to the northwest of Vancouver, British Columbia (B.C.) (Figure 1.1). The shores of the fjord are mostly steep-sloped bedrock with the occasional cobble/pebble beach (Macdonald *et al.* 1992). At the northern point of Howe Sound, the Squamish River enters the sound. The overall sound basin is separated by a shallow sill near Porteau Cove into two major sections: the northern basin and southern basin. The northern basin of Howe Sound contains Darrell Bay, Britannia Beach and Porteau Cove while the southern basin consists of several islands that gives rise to two major channels; the west channel and the east channel. The west channel consists of Lion's Bay and the east channel consists of Chaster Bay and Port Mellon (Figure 1.1). There were three main anthropogenic sources that led to ecological disruption at Howe Sound: (1) The Woodfibre pulp and paper mill, (2) the Britannia mine copper mine found in the Northern basin, and (3) the Port Mellon pulp and paper mill along the eastern channel (Figure 1.1).

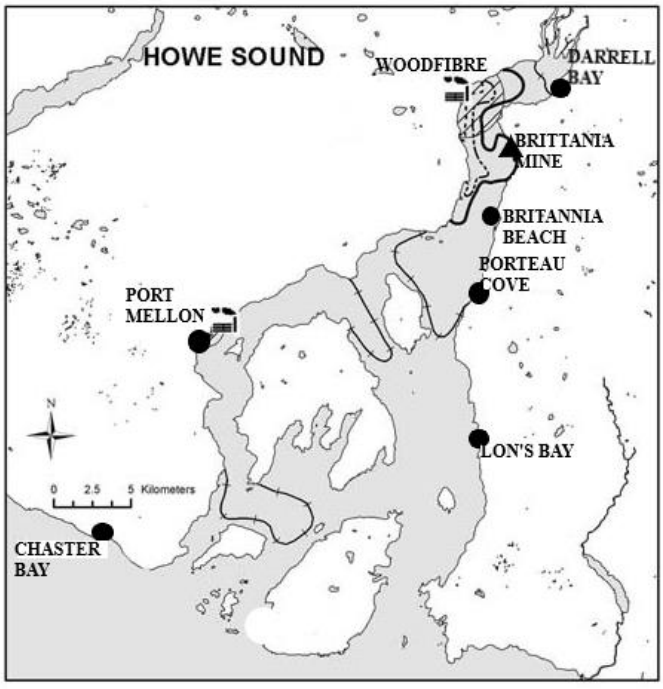

Figure 1.1. Locations of the six study sites surveyed in Howe Sound (B.C, Canada) Pulp and paper mill history

The Woodfibre pulp and paper mill (PPM) was located at the north head of Howe Sound. The Woodfibre PPM started its operation in 1912 and was permanently closed in 2006. While in operation, it contributed largely to the contamination of Howe Sound. In 1912, the mill employed the use of a calcium sulphate cooking process to produce its pulp, and then switched to elemental chlorine and hypochlorite in 1938 (Haggen, 1923). The use of calcium sulphate resulted in the formation of various bases such as ammonium, calcium, sodium hydroxide etc. (Lenzing *et al.* 1997). From 1958 until the mill's closure in 2006, the mill produced its pulp through Kraft bleaching, which uses chlorine containing compounds to produce pulp from wood chips (Munkittrick *et al.* 1991 Sreekrishnan & Ali, 2001).

During operation at Woodfibre PPM, some series of events occurred which contributed to the pollution in Howe Sound: (1) an explosion of a pulp boiler in 1963, which led to spillage of pulp into nearby Darrell Bay (no documented amount of pulp spillage or cause of explosion) (England, n.d.); (2) flooding in 1963 which led to the wash off of wood chips and other pulp wastes into Darrell Bay (Squamish-Library, n.d.); and (3) collapsing of a dock and warehouse at the mill in 1955 which led to the spillage of about 1400 tons of pulp (Squamish-Library, 2011).

The Port Mellon PPM started its operation in 1909, shut its paper production in 2016 due to financial difficulties but still continues to produce pulp till this day. In 1909, the mill started its operation using soda pulping and switched to unbleached Kraft pulping in 1916. In 1954, the mill switched again, this time to a semi-bleached pulp production which was soon followed by full bleached pulping from 1962 until closure of the PPM (Willems, 2004). The use of semi- and full-bleached pulping increased concentration of adsorbable organic halides (AOX), dioxins and furans in Howe Sound. In 1989, the Port Mellon PPM began to use elemental chlorine in place of chlorine dioxide, thereby reducing the level of AOX released into Howe Sound (Willems, 2004).

1.1.1. Pulp and paper mill environmental contamination

The PPM industries in Howe Sound contributed to environmental pollution through its series of processing and production (Pokhrel & Viraraghavan, 2004). Both pulp mills deposited large amounts of wood fibre, chips and debris into the sound, these

solid wastes created fibre beds or mats around its outfalls which extended several hundred meters around Howe Sound (Hatfield, 1994). Dioxins and furans were produced by the pulp mills in large quantities before substantial technological modifications (such as dredging) were initiated in the early 1990s (Hatfield, 1994). Severely depressed subtidal and intertidal communities were documented near both pulp mills (Hatfield, 1994). Effect of the pulp mill effluents was estimated to extend many kilometers around the outfalls. A thick mat of pulp fibers (>10 cm) has been observed 6 km away, on the Darrell Bay shore after storm events (Vassilenko, unpublished data).

Log storage is another major source of disruption in Howe Sound. Light attenuation, accumulation of wood waste, chlorophenolic compounds, dioxin and furans, resin acids and creosote in sediments are some of the effects of log storage. Some resin acids, such as dehydroabietic acid are highly persistent and are being produced in large quantities, which make them to be the most commonly found resin acids in receiving waters affected by pulp mills effluents and log storage (Pokhrel & Viraraghavan, 2004).

1.2. Britannia mine history

The Britannia copper mine was the world's leading copper producer in the British Empire from 1904 to 1974. The mine was closed due to the inability of the company (Anaconda Canada Ltd) to maintain production cost (McCandless, 2015). During its time of operation, Britannia mine processed approximately 5500 tons of copper per day from several underground headings within Britannia (Haggen, 1923). In 1970, the mine was issued a complaint by the Director of Pollution Control with regards to contamination of freshwater fisheries and the water supply, but no action was taken (McCandless, 2015). In 1996, it was estimated that approximately 400 kg of copper, 150 kg of zinc and 1.4 kg of cadmium passed into Britannia Creek and into Howe Sound per day (Levings *et al.*, 2004).

The contamination of freshwater fisheries and water supply at Britannia mine were regarded as unmanageable due to three main factors: (1) the rocks at Britannia mine, (2) the structure of the mine and (3) the climatic setting within the mine area (McCandless, 2015). The ores found within granitic rocks at Britannia Mine contained high concentrations of metallic sulphide minerals such as iron pyrite (McCandless,

2015). Exposure of these metallic sulphide minerals to water and air, fosters the formation of weak acids, which can dissolve other minerals (i.e., iron and manganese) and release heavy metals, such as copper, zinc and cadmium into receiving waters (McCandless, 2015). The heavy metals and sulfuric acid entering receiving waters form acid rock drainage (ARD) (McCandless, 2015).

The structure of the mine is quite different when compared to typical mines. Most mines have a vertical excavation tunnel which is drilled from the top of the soil to the bottom where the ores are located; however, Britannia mine had horizontal excavation tunnels, with two tunnels located at the sides of the mountain (a higher 2200-exit portal and lower 4100-exit portal (Figure 1.2) (McCandless, 2015). The 2200-exit portal paved an exit route for AMD to flow from the underground ores to Britannia beach at lower elevation while the lower 4100-exit portal drained directly into Howe Sound through an exit outfall.

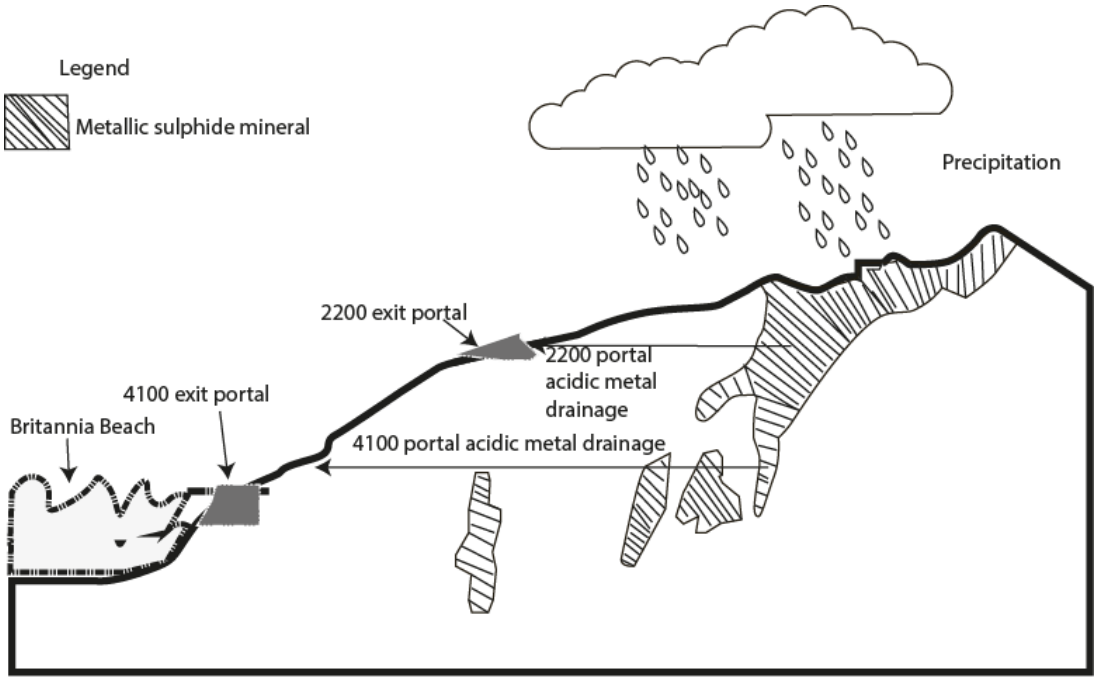

Figure 1.2. Schematic diagram of Britannia mine structure
The structure of the Britannia mine with exit portal 2200 and 4100 exit portal. The 4100 exit portal released wastewater into Howe Sound while the 2200-exit portal directly released waste water onto Britannia Beach (Smitheringale, 2011).

The last factor promoting the contamination of freshwater fisheries and the water supply at Britannia is the climatic setting in the mine area. The Northern basin of Howe Sound receives about 2400 ml of precipitation each year (McCandless, 2015). All

precipitation falling into the mine aids in the formation of ARD, which exits the underground mine through both the 2200 and 4100 exit portals (McCandless, 2015). The spring and fall seasons are peak periods for ARD due to melting snow and increased rainfall (Levings et al., 2004)

1.2.1. Britannia copper mine environmental contamination

Britannia mine deposited significant sulfide tailings into Howe Sound during its operation from 1904 to 1974. Mine tailings were concentrated within 3 km of the shore slope of Britannia Beach and covered most of the inner basin floor (Hagen, 2004). In the late 1990s, subsurface layers of tailings continued to generate ARD (ARD) (Grout & Levings, 2001). The formation of ARD results from the autocatalytic reaction between water, oxygen and bacteria (Peppas et.al., 2000), along with pyrite and metals from the tailings, ore stock piles, mine pits and waste rock deposits (Salomons, 1995). ARD is characterized by low pH (2-5) and high concentration of heavy metals (e.g. copper, iron, zinc) which may result in proton acidity and/or mineral acidity (Johnson, 2003). At the Britannia copper mine, spring and fall seasons are the peak periods for AMW discharge as mentioned above (Levings *et al*., 2004). ARD from portal exit 4100 was discharged directly into Howe sound until 2006 when a water treatment plant was built (McCandless, 2015). Despite the mine closure and ARD treatment, elevated levels of metals were still present in water and sediments at Britannia Beach and areas at least 5 km south from the mine discharge (Hagen, 2004). Previous studies have shown that metal concentrations near Britannia Beach were elevated up to three orders of magnitude compared to Porteau Cove (Hagen, 2004). For example, the level of copper at Britannia Creek was 1,200 mg/kg sediment, while at Porteau Cove it was 18 mg/kg sediment (Hagen, 2004). Metals such as copper, lead and zinc were present in sediments above potentially toxic levels (Hagen, 2004). Along with ARD, solid wastes were recovered from the copper mine which existed in several different forms including: metallic waste (heavy, medium, and fine scrap), compound waste (oxide scale), oxidized powered waste (e.g. anode slime, pickling sludge, floor dust, flue dust, spent catalyst, effluent sludge and mine tailings), and oxidized bulk waste (slag and dross)(Agrawal et al., 2004). Some of the aforementioned forms of wastes contained high levels of heavy metals such as lead, zinc, iron copper and manganese (Agrawal et al., 2004).

1.3. Biomarkers

In Howe Sound, the cessation of some industrial activities has occurred over the last few years, and technological modifications have decreased contaminant input into Howe Sound from currently active sources. Nevertheless, the extent of historical contamination and the cumulative effects on biota in Howe Sound are currently unknown. To better understand the effect of environmental contaminants, present at Howe Sound, a set of biomarkers were employed in this study. Biomarkers generally involve the measure of biochemical, cellular or physiological parameters as a diagnostic tools for detecting stress in an organism or community, and to detect exposure to specific contaminants (McCarthy & Shugart, 1990). According to two studies (Lagadic et.al.,1997; Cossu-Leguille & Vasseur, 2003), a complete set of biomarkers should encompass the following: (1) for a biomarker to serve as an early indicator, its response should be sensitive to pollutant exposure and effects; (2) when using a biomarker, its baseline value should be well-defined in order to differentiate a natural variability from pollutant induced stress; (3) any confounding factors that might affect the biomarker response should be well understood or known; (4) the relationship between biomarker responses and pollutant exposure should be identified and understood; and (5) the link between biomarker response and long term impact on organisms should be established. There are three main types of biomarkers: biomarkers of exposure, biomarkers of effects, and biomarkers of susceptibility.

Biomarkers of exposure are used to assess the amount of pollutant and/or its metabolites that are present within the body of an organism (Cossu-Leguille & Vasseur, 2003). These pollutants can be measured in blood, saliva, urine and if soluble may be measured in body fat and breast milk. Biomarkers of exposure are the most widely used since they can provide information on route, pathway and sometimes even the source of exposure (Cossu-Leguille & Vasseur, 2003). These indicators allow researchers to work forward in time to determine an exposure and prevent it from causing further damage. Biomarkers of effect are indicative of biochemical or other changes within an individual organism due to exposure of a pollutant (Timbrell, 1998). These changes may include modifications in blood composition, alteration of specific enzyme activities, the appearance of DNA adducts, and increase in mRNA or protein levels, etc. (Timbrell, 1998). Biomarkers of susceptibility serve as indicators of particular sensitivity of

organisms to the effect of a pollutant (Timbrell, 1998). In this study, two types of biomarkers of exposure were examined, which will be explained further in the next section.

1.3.1. Biomarker of exposure

Biomarkers of exposure are indicators that reveal contact with pollutants through physiological property alterations in a biological model organism that may also help explain the toxicodynamics of the pollutant (Cossu-Leguille & Vasseur, 2003). Biomarkers of exposure indicate an exposure to a compound and its extent, which is observed by measuring a potential marker of that compound or its metabolite in body tissues and/ or fluids (Timbrell, 1998). The measurement of the internal dose within a body is essential since organisms vary in rates of absorption, distribution, metabolism and excretion (Timbrell, 1998)

One of the two main biomarkers of exposure used in the present research project was metallothionein. Metallothionein (MT) is a cysteine-rich metal binding protein with low molecular mass (Roesijadi, 1992c). MT is made of different groups of metalloproteins with a detailed sequence of amino acid residues that are sulfhydryl-rich such as cysteine. MTs have the ability to bind to various metals, which can be essential (e.g. Zn^{2+}, Cu^{2+}) or non-essential (e.g. Cd^{2+}, Hg^{2+}, Ag^{+})

MTs are mostly concentrated in the liver of aquatic vertebrates, but they can also be found in kidney, gills or digestive tissues in invertebrates. Aquatic vertebrates and invertebrates may take up metals through their gills (in the case of dissolved form of the metals) or through the digestive tract (with ingested food) (Leland and Kuwabara 1985). MT plays a role in maintaining the homeostasis and detoxification of metals *via* a multi-stage process. As shown in Figure 1.3, the induction of MT messenger RNA (mRNA) begins at the MT gene with a metal ion cation (ME^{2+}) binding to a metal regulatory factor (MRF). This regulatory factor undergoes conformational changes to expose the metal regulatory element (MRE) that is required to induce the transcription of MT mRNA. The induced MT mRNA is translated in order to give rise to MT protein, so it can rapidly sequester free metal cations and promoting detoxification (Roesijadi, 1992) (Viarengo et.al., 1999).

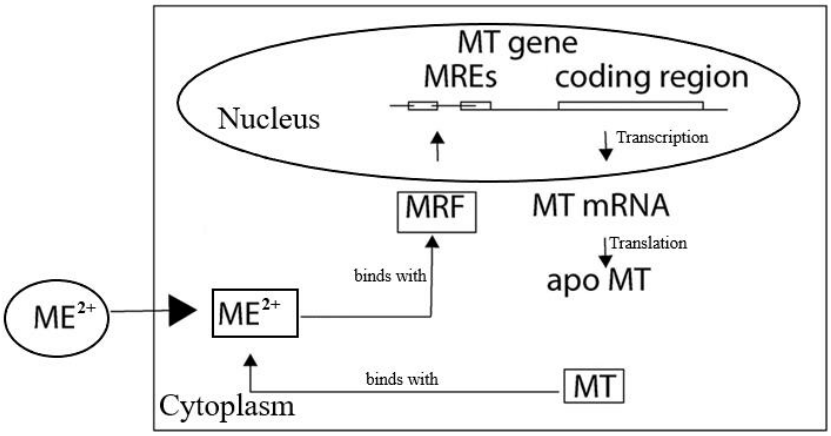

Figure 1.3. Schematic diagram of induction of metallothionein in a eukaryotic cell following exposure to metal (ME^{2+})

Source: Roesijadi, 1992a)

The second biomarker of exposure that was implemented in this current project was ethoxyresorufin-O-deethylase (EROD) activity. This biomarker of effective dose exposure provides evidence of induction of the CYP1A subfamily by xenobiotics such as planar halogenated hydrocarbon and polycyclic aromatic hydrocarbons (PAHs), dioxins, furans and organochlorine pesticides, biphenyls etc. EROD activity is part of a complex cascade reaction, which involves induction of cytochrome P450-dependent monooxygenase (or P4501A) in response to xenobiotic stress. This activity is measured as the rate at which cytochrome P4501A is able to catalyse the deethylation of 7-ethoxyresorufin (7-ER) to resorufin. Aromatic compounds such as PAHs are known to induce the production of cytochrome P4501A with the assistance of heat shock proteins (HSPs). These proteins (HSPs) are upregulated in response to a wide range of xenobiotic stressors, such as PAHs (McCarthy & Shugart, 1990). One particular protein, HSP 90, is induced by a variety of stressors including heavy metals, salinity and oxidative stress. As seen in Figure 1.4, induction of cytochrome P450 1A begins with the binding of aryl hydrocarbon receptors (AHR) aromatic hydrocarbons. This action promotes the release of HSP 90 which starts a signal transduction cascade that mediates the binding of AHR to the aryl hydrocarbon translator protein (ARNT). The heterodimer formed by both the AHR and the ARNT leads to the induction of cytochrome P4501A (Whyte et.al., 2000).

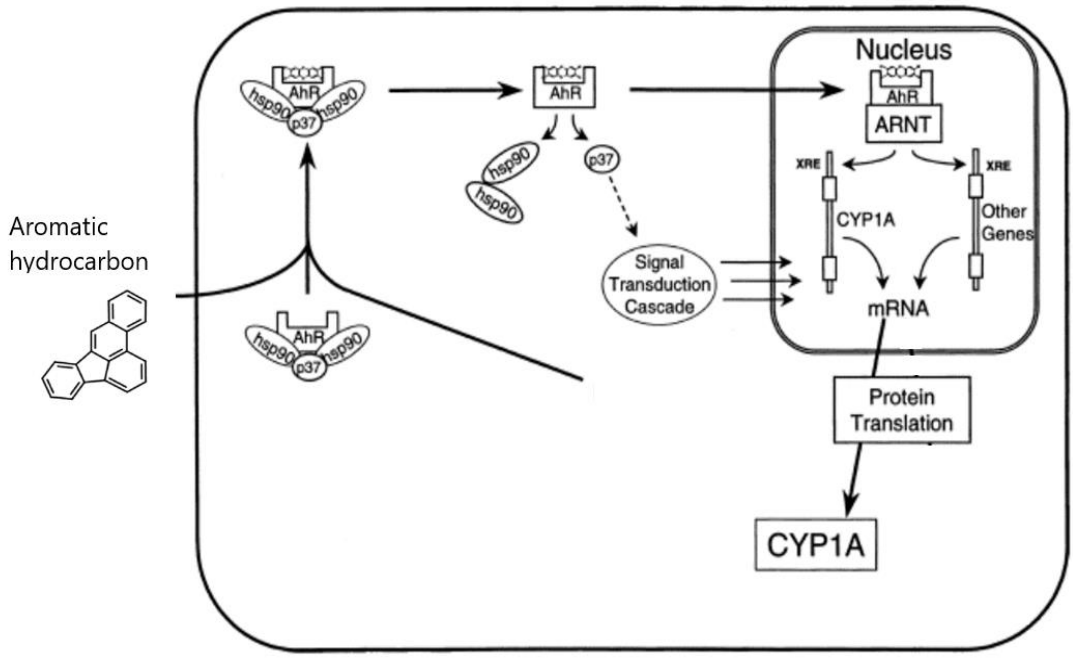

Figure 1.4. Schematic diagram of induction of cytochrome P450 1A by AhR active compound.

Aromatic hydrocarbon compounds such as PAH, binds to the aryl hydrocarbon receptor, this initiates the production of CY1A1. CYP1A1 then converts 7-ethoxyresorufin to resorufin which is measured fluorometrically (Mohammadi-Bardbori, 2014).

1.4. Bioindicators

Another term used frequently in environmental assessment is bioindicator, which are organisms that provide information through their presence or absence in the environment, or through their behaviour or physiology in an environment (McGeoch et.al., 2014). Bioindicators are divided into three groups; environmental indicators, ecological indicators and biodiversity indicators, but only two groups (ecological indicators and environmental indicators) were employed in this study.

Mussels were one of two ecological indicators used in this study. Levels of MT and EROD activity were assessed to monitor the physiochemical changes that occurred at Howe Sound as a result of industrial activities. When using an ecological indicator in a complex ecosystem like Howe Sound, multiple factors such as variability in pH and salinity, as well as availability and concentration of multiple pollutants, may pose a threat to accurately assessing the effect of contaminants within an ecosystem. The use of mussels as an ecological indicator allow for the evaluation of performance of aquatic organisms within each monitoring site (Hodkinson & Jackson, 2005). Also, the use of

mussels as an ecological indicator span from its the long-term stability in assessing habitat continuity, abundance in the community and the ability to filter feed (Hellawell, 1986; Norden & Appelqvist 2001).

Giant kelp (Macrocystis pyrifera) was the second ecological indicator used in the present study. This organism is a canopy-forming alga, which forms extensive submarine forests, a habitat for a diverse and abundant aquatic community (Anderson & Hunt, 1988) Monitoring the early stages of giant kelp provides understanding to the effect of various contaminants present in the water column (Anderson & Hunt, 1988). Marine alga naturally bioaccumulate metals from their surrounding environment. Uptake of metals occur through rapid passive transport, followed by a slower active transport (Bates et.al 1982). During passive uptake, metals are absorbed through the cell surface within minutes (Bates et.al 1982). As active transport begins to advance, the cell membrane transports metal ions into the cytoplasm (Bates et.al 1982). Transport of heavy metals to the cytoplasm fosters the binding of metal ions to intracellular compounds (such as cytoplasmic ligands and phytochelatins), leading to intracellular precipitation (Kadukova & Vireikova, 2004). This may cause the inhibition of growth, prevent transport of compounds through cell walls, and ultimately result in mortality of the Giant kelp (Evans & Edwards 2011).

Finally, an intertidal assessment was used as the environmental indicator in this study. The whole community assessment includes the evaluation of species richness (the number of species present), the relative abundance of different species, and the presence of keystone species (species having other species that might depend on them) (McGeoch, 1998). Whole community assessment may be used as an environmental indicator to monitor the overall state of an ecosystem (Aubry & Elliott, 2006). The use of intertidal diversity to better understand the current state of an aquatic environment originates from the ability of these intertidal species to sustain a community. The presence and absence of certain intertidal species may point or express the instability in an environment (Gaufin et al.,1952). The intertidal community consist of mostly microorganisms and macroinvertebrates. Microorganisms such as encrusting algae and micro-bacteria etc. may serve the ecosystem by providing organic materials as food for other species in the ecosystem (Gaufin et al., 1952). The inclusion of macroinvertebrates in intertidal assessment have shown to provide evidence of contamination in an environment. For example, gilled aquatic invertebrates may be affected in a

contaminated environment due to reduction in dissolved oxygen in water (Goodnight, 1973).

1.5. Objective of research

The overall objective of this study was to examine the biological effects of historical and on-going contamination for six selected sites at Howe Sound, which are categorized as high, moderate and low exposure sites. Each assay/test carried out in this research was used to assess the effects of historical industrial processes within this ecosystem. The two biomarkers of exposure (MT and EROD) were specifically selected and measured in mussels to assess the presence and bioavailability of contaminants that might still exist at these study sites. Intertidal biodiversity assessment as well as 48-hour giant kelp (*Macrocystis pyrifera)* percent germination and tube length tests were selected to assess the total effect of these contaminants at the same study sites. Results from the assays/tests were compared between the high, moderate and low exposure sites in order to see if there is a strong correlation between the degree of environmental contaminant exposure at Howe Sound and the level of adverse effects on ecosystem health.

Chapter 2. Materials and Methods

2.1. Study sites

To assess the biological effects of historical and ongoing contamination in Howe Sound, six sites were selected (Darrell Bay, Britannia Beach, Lion's Bay, Porteau Cove, Port Mellon and Chaster Bay) for study. The sites were categorized as either high, moderate, or low exposure sites based on their proximity to three main points of contamination; the Woodfibre PPM, the Port Mellon PPM, and the Britannia copper mine. All sites selected were geographically similar with rocks at least 15-50 cm in diameter at the mid to low intertidal level (0.5-1 m) and having low-sloped beaches which were protected from direct wave action.

The geographical locations of each site within Howe Sound and the nearest source of contamination to each was listed in Table 2.1. Selected sites were further classified based on the historic exposure to pulp and paper mill effluent using two tracer parameters: pulp fibres and organochlorine compound concentrations measured around Howe Sound in 1990 and 1991 by Shannon Bard (Bard, 1998). Accordingly, three pulp and paper mill effluent exposure zones were identified: high, moderate and low. The high exposure zone was affected by both pulp fibres and organochlorines and extended up to 10 km from both PPM outfalls in the 1990's (Bard, 1998). Pulp fibres did not affect the moderate exposure zone, but its sediments were contaminated with organochlorines at levels higher than 10 ppm (Bard, 1998). In the 1990's, the moderate exposure zone extended from 10 to 25 km from the PPM outfalls. Beaches that were unlikely to be exposed to the mill effluents were defined as low-exposure zones (Bard, 1998).

Six sites were selected in this study; Chaster Bay was designated as the low exposure (reference) site. Porteau Cove and Lion's Bay were chosen as moderate exposure sites, and Port Mellon, Darrell Bay and Britannia Beach were selected as high exposure sites. Darrell Bay is located in the north of Howe Sound directly adjacent to the former Woodfibre PPM. Darrell Bay served as a ferry dock to Woodfibre PPM until 2006. In close proximity to Darrell Bay is Britannia beach which is located beneath Britannia mine. Porteau Cove is located at the eastern shore of Howe Sound approximately 10 km and 24 km south of Britannia Mine and Darrell bay, respectively. Lion's Bay is about 20 km and 35 km south of Britannia Mine and Darrell Bay, respectively. Port Mellon is

located on the western shore of Howe Sound, and in immediate proximity to the Port Mellon PPM. Chaster Bay which located in Gibsons on the lower portion of Sunshine Coast, is the reference site for this study.

Table 2.1. Seven Howe Sound sites were surveyed in this study. The sites were classified based on Bard's study (1998) according to their exposure to the pulp mill effluents in 1990.

Study Site	Nearest source Pulp mill	Exposure rating
Chaster Beach	Port Mellon PPM	Low Exposure (reference site)
Lion's Bay	Woodfibre PPM, Britannia Mine	Moderate Exposure sites
Porteau Cove	Woodfibre PPM, Britannia Mine	
Britannia Beach*	Woodfibre PPM, Britannia Mine	High exposure
Darrell Bay	Woodfibre PPM	
Port Mellon	Port Mellon PPM	

2.2. Mussels

Adult mussels (*Mytilus trossulus*) were collected from the intertidal zone at each site in June 2015 during low tide periods. Mussel size varied for length (5.0 ± 1.7 cm), width (3.0±1.5 cm) and height (2.5 ± 1.0 cm). Mussels were kept cold and transported to the laboratory on the same day of harvesting. For each mussel, digestive glands were harvested and 0.18 ± 0.03 grams was immediately frozen on dry ice and were stored in -80 ^{0}C freezer for subsequent analysis.

2.3. EROD assay

Homogenization buffer was prepared using 0.150 M KCl, 0.001 M EDTA, and 0.001 M DTT. Reaction buffer consisted of 0.1 M phosphate buffer using KH_2PO_4 (pH 7.4), 0.019 M NADPH and 0.0025 M of 7-ethoxyresorufin (purchased from Santa Cruz Biotechnology Inc., Dallas, TX, USA). The standard resorufin salt used for the standard curve was purchased from Sigma Aldrich (St. Louis, MO, USA).

Ethoxyresorufin-O-deethylase (EROD) activity was measured using mussel digestive gland tissue. The procedure used to measure this activity was adopted from Burke and Mayer (1974). Briefly, mussel digestive gland tissue was homogenized in homogenization buffer (1:5, w:v, buffer composition at pH 8.0) at 30 rpm for 6 min using a Mixer mill MM300 homogenizer (Retsch. Newtown, PA, USA). The resulting homogenate was centrifuged for 30 min at 12000 × *g* at 3 ^{0}C using a refrigerated microcentrifuge 18R (VWR International, Radnor, PA,USA). The resulting supernatant was then used to analyze EROD activity.

The supernatant was mixed with four volumes of reaction buffer (pH 7.4), and 200 µL this mixture was incubated in each well of a 96-clear well microplate at 30 ^{0}C for 1 h. The enzymatic reaction was terminated by adding 50 µl of 0.1 M NaOH. Fluorescence was measured on a Spectra Max M2e microplate reader (Molecular Devices. Sunnyvale, CA, USA) at 520 nm excitation wavelength and 595 nm emission wavelength. Resorufin salt solutions of known concentrations ranging from 74 to 2375 µM were included on each microplate and used to generate a standard curve for resorufin. EROD activity was normalized to total protein content after measurement using Bradford assay (Bradford, 1976). Bovine Serum Albumin (BSA) solutions of known concentrations ranging from 0.03 to 1 mg/ml were included on each plate to generate standard curve for proteins. EROD activity was expressed as µmoles resorufin / h / mg total protein.

2.4. MT assay

MT assay was adapted from (Viarengo & Burlando, 1999). Homogenization buffer was prepared using 500 nM sucrose, 200 mM Basic TRIS, 100 mM phenylmethylsulfonyl fluoride (PMSF) and 0.01% β-mercaptoethanol (added at the time of experiment). Both PMSF and β-mercaptoethanol were purchased from Sigma Aldrich (St Louis, MO). Working buffer was prepared using 200 mM of dibasic sodium phosphate ($Na_2HPO_4.7H_2O$) and 0.43 mM of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) purchased from Sigma Aldrich (St Louis, MO).

Digestive gland tissues were homogenized in homogenization buffer (1:5 ratio, w:v, buffer composition) using Mixer mill MM300 homogenizer (Retsch, Newtown, PA, USA) at 30 rpm for 6 min. The resulting homogenate was centrifuged at 120,000 × *g* for 1 hour. In a new microfuge tube, 250 µl of the resulting supernatant, 265 µl of 100 % ethanol (cooled at -20 ^{o}C) and 20 µl of chloroform (cooled at -20 ^{o}C) were mixed by vortex and then centrifuged at 6000 × *g* for 10 min at 4 ^{o}C. 300 µl of the resulting supernatant, 20 µl of 37% HCl and 900 µl of cold 97% ethanol were mixed by vortexing, before incubating at -20 $^\circ$C for 1 h. After incubation, the supernatant was centrifuged at 20,000×*g* for 30 mins. The supernatant was then discarded, and the resulting pellet was washed using 800 µl solution containing 87% of ethanol, 1% chloroform and 12%, of basic Tris buffer (20 mM). The pellet was further mixed with the solution by vortexing,

and then centrifuged at 20,000 × *g* for 30 mins. The solution was discarded and the pellet was dried under nitrogen gas (Viarengo & Burlando, 1999).

To measure MT concentration, 150 µl of NaCl (250 mM) solution and 150 µl of EDTA-HCl solution were added to the pellet and mixed by vortexing. Approximately 100 µl of the resulting sample and 1400 µl of working buffer were transferred into a new microfuge tube, vortexed before pipetting 200µl onto a 96 well microplate (Viarengo & Burlando, 1999). MT concentration for each sample was estimated using reduced glutathione (GSH) standard curve of 15 µM to 120 µM assuming that 1 molecule of metallothionein contains 20 molecules of protein cysteine content. Metallothionein activity for each sample was normalized to the total protein content using Bradford assay (µg MT/ mg total protein). Bovine serum albumin is commonly used to determine the quantity of other protein by comparing an unknown concentration of protein to a known amount of BSA concentration. Bovine γ-globulin at a concentration of 1 mg/mL in working buffer was used as a stock solution. BSA concentration was further diluted using working buffer at concentrations; 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.0625 mg/ml and 0.0313 mg/ml (Bradford, 1976)

2.5. Intertidal species richness

Points of quadrat sampling were restricted to areas with bands of mussels. The diversity of intertidal species was analyzed using one 3 m × 3 m quadrat per site as described in Bard (1998). Each 3 m × 3 m quadrat was sub-divided into 0.5 m × 0.5 m sections using a wooden quadrat frame, which created a 36-quadrat grid. The species richness was estimated for each site by flipping rocks and species observed under and on top of rocks were identified and counted manually. The intertidal species richness analysis was carried out in the months of June, July and August in 2015 and only data collected at this time were used to assess intertidal species richness

2.6. Giant kelp (Macrocystis pyrifera) 48-h germination and germ-tube growth test method

Two liters of water were collected at each study site during low tides at the shores using a 5 litre container with lids. These water samples were used to conduct a static non-renewal 48-h giant kelp germination and tube growth test (Anderson & Hunt,

1988). All control and test solutions were performed in triplicate. Giant kelp specimens used for this test were supplied by Kim Siewers (Santa Cruz, CA).

Protocol for the Giant kelp and tube length assay was adapted from Anderson & Hunt, 1988. *Macrocystis* zoospores were obtained from the reproductive blades (sporophylls) of the adult giant kelp as follows; sporophyll blades were soaked in filtered sea water for 20 min, removed, dabbed with paper towels and allowed to air dry for 1 h. Next, sporophyll blades were placed back into the filtered seawater for 10 min to stimulate zoospore release (cloudiness of water was indicative of zoospore release). The resulting spore mixture was allowed to settle for 30 min. Spore density was estimated by fixing 9 ml of the spore mixture solution with 1 ml of formalin before counting with a bright-line hemacytometer.

All spore mixtures was incubated in a petri dish for 48 h at 15 ± 1 ^{o}C under cool white fluorescent lights (50 ± 10 µE/m^2/s) and salinity adjusted to 34 ± 2 using brine. After 48 h, the test was terminated by fixing each sample with 1 ml of 37% of formalin. 100 germinated zoospores was counted using an electron microscope (OMAX 3.5X-90X), and 10 zoospores were randomly picked and their tube length was measured using ImageJ (Wayne Rasband).

2.7. Statistical analyses

Data analyses for EROD and MT assays, as well as the giant kelp germination and tube growth length test was based on a randomized block design. All data were first tested for normality using Kolmogorov-Smironv test and homogeneity using Cochran C test. Both MT and EROD data results did not pass the normality test, and therefore both MT and EROD data were subjected to natural log transformation (log 10) before further be analyzed by a one-way analysis of variance (ANOVA). The mean difference between experimental groups were considered statistically significant at $P < 0.05$. The Tukey Honesty Significant Difference (HSD) post-hoc test was employed to statistically differentiate the mean values between each site. ANOVA and Tukey tests were carried out using Sigma plot (Systat software, Inc. San Jose, CA).

Data analysis for intertidal species richness was conducted using the frequency of different species that occurred at individual sites. The result was calculated as a percentage of the total species obtained from all study sites.

Chapter 3. Results

3.1. EROD assay

EROD activity at Britannia Beach was statistically significantly higher compared to the control site (Chaster Bay) (Figure 3.1). The increased mean EROD activity observed at Britannia Beach was in accordance with the source of contaminant found at this site (Grout & Levings, 2001). EROD activities for mussel digestive gland tissues collected at moderate exposure sites (Porteau Cove and Lion's Bay) were not statistically significantly different from each other; however, the activities from these sites were significantly higher than the control site (1.9 and 1.6 fold increase respectively) (Figure 3.1).

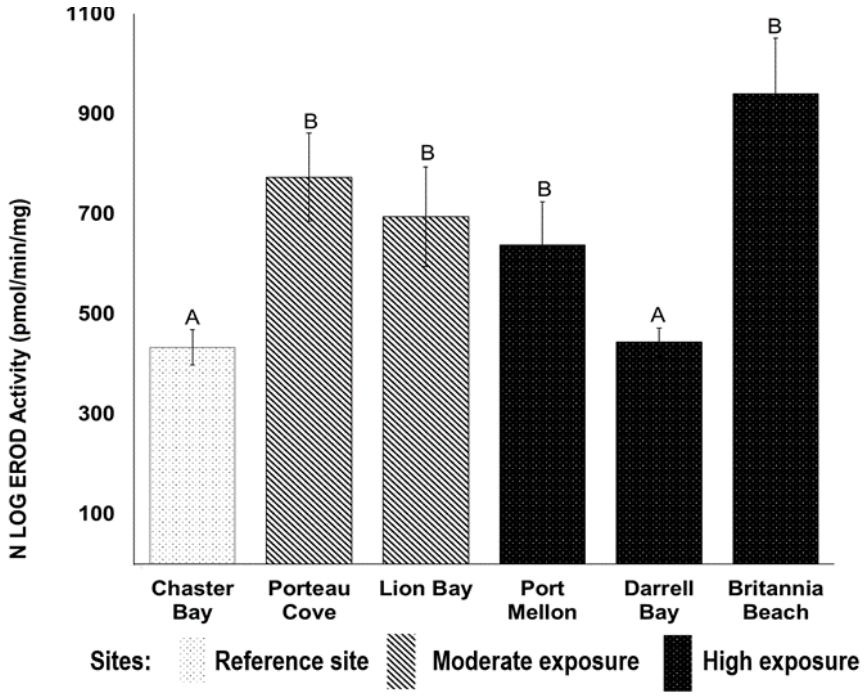

Figure 3.1. EROD activity across different study sites at Howe Sound
Measured EROD activity in mussel digestive glands collected from the study sites. Sites were grouped according to the pulp mill exposure classification according to Bard (1998) with the exception of the Britannia mine site. Data is represented as means ± SEM (n=6). Simlar letters represent EROD activities that are not statistically different to each other ($p<0.05$).

3.2. Metallothionein (MT) assay

A significant increase in MT was found in mussel tissue collected at one of the moderate sites, (Porteau Cove) and at two of the high exposure sites (When compared t the reference site, Darrell Bay was ~ 2.8-fold higher, and Britannia Beach ~4.2-fold higher.

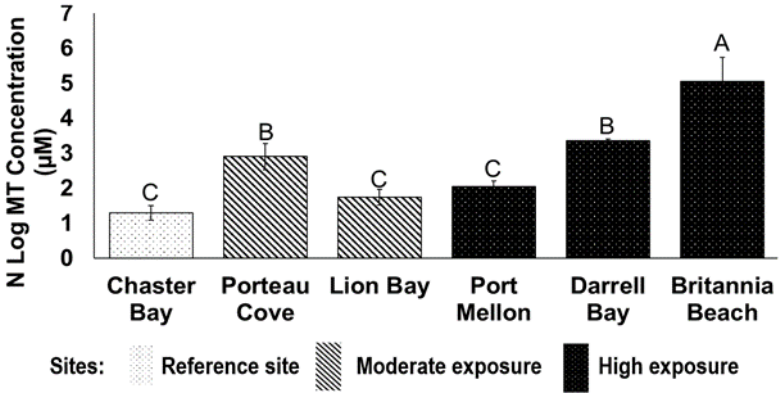

Figure 3.2. Metallothionein (MT) concentration in mussel digestive glands collected from various study sites in Howe Sound.
Sites were classified according to Bard (1998). Different letters represent MT concentration in mussel tissue that are statistically different from each other ($p<0.05$).

3.3. Giant kelp germination and tube length

The percent mean germination rates were determined for giant kelp incubated in water collected from each study site (Figure 3.3). The only sites that showed significantly lower percent germination rates compared to the reference site were Lion's Bay (moderate exposure) and Port Mellon, Darrell Bay and Britannia Beach (high exposure sites). These first three sites had greater than a 45% decrease in percent germination rates while Britannia Beach had an approximate 16% decrease (Figure 3.3). In addition to germination rates, the mean germination tube length was also measured in giant kelp. With exception of Porteau Cove and Port Mellon, a statistically significant decrease in germinated tube length were found at Lion's bay (~20% decrease), Darrell Bay (~37% decrease) and Britannia Mine (~10% decrease) when compared to the Chaster Bay reference site (Figure 3.4).

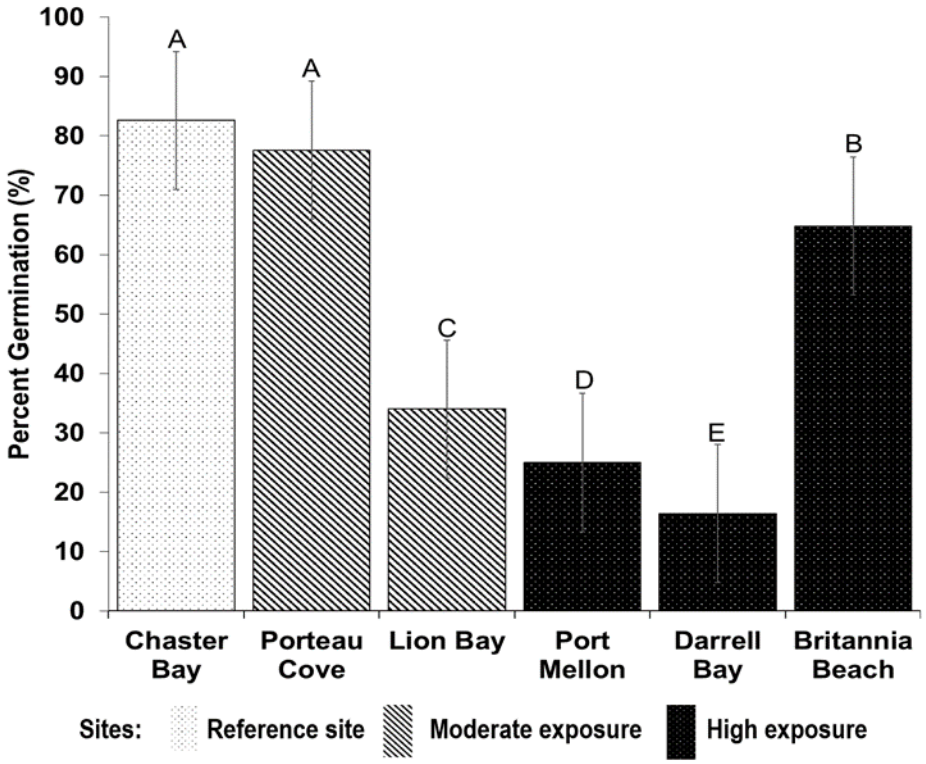

Figure 3.3. Giant kelp (*Macrocystis pyrifera*) germination test.
Giant kelp zoospores incubated in water samples collected from each site in Howe Sound; a brine saltwater treatment was used as a negative control. Data is represented as total mean percentage of spores germinated. Similar letters represent germination percents that are statistically similar to each other ($p<0.05$).n=100

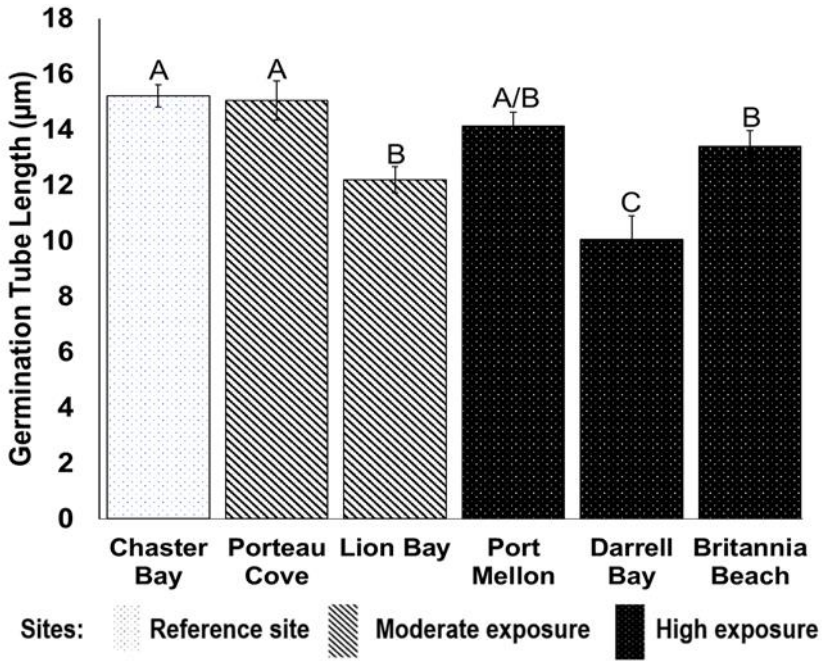

Figure 3.4. Giant kelp (*Macrocystis pyrifera*) germination tube length.
The effect of site water on the germination tube length of giant kelp. Similar letters represent germination tube lengths that are statistically similar to each other ($p<0.05$).n=10

3.4. Intertidal species diversity

Results obtained from the intertidal species diversity assessment showed that Chaster Bay (reference site) had the largest diversity and contributed 36.9% towards the total species observed in Howe Sound. The result for the moderate exposure sites (Porteau Cove and Lion's Bay) contributed 17.78 % and 19.02 % to the total species observed, respectively. The results from high exposure sites (Port Mellon, Darrell Bay and Britannia Beach) contributed 12.79 %, 4.50 % and 8.56 % respectively. The tolerance of species across different study sites were not statistically different (19.692 likelihood ration (x^2) p= 0.0962).

Chapter 4. Discussion

Although cessation of many industrial activities has occurred, and technological modifications have decreased point source industrial inputs into Howe Sound. Historical data on the intertidal community biodiversity indicated that recovery of the ecosystem has been minimal, particularly at heavily impacted sites (Darrell Bay, Britannia Beach and Port Mellon) when compared to less impacted sites (Lion's Bay, Porteau Cove and Chaster Bay) as seen in the summary table 4.1 below. The goal of this study was to assess the present chemical and biological conditions at intertidal areas with different levels of exposure to pulp mill effluents and copper mine waste discharge. In addition, the present study evaluated the potential effects of these contaminated sites in Howe Sound. To do so, the use of various biomarkers and bioindicators were employed. For bioindicators and biomarkers, mussels, giant kelp and the intertidal community assessment were used in this assessment.

Table 4.1. Summary of endpoints performed at Howe Sound sites. For all tests, sites statistically signicantly different from the refrence site (Chaster Bay) is classified as either moderate, high, very high or extremely high

Site	Exposure rating	Nearest source of contamina-tion	EROD activity	MT assay	Reduction in Giant Kelp germina-tion	Reduct-ion in Giant Kelp tube length	Reduct-ion in intertidal species richness
Chaster Bay	Low exposure (reference site)	None	low	low	low	low	low
Britannia Beach	High Exposure	Britannia copper mine	moderate	high	moderate	moderate	high
Darrell Bay		Woodfibre pulp and paper mill	low	moderate	high	high	high
Port Mellon		Port Mellon pulp and paper mil	moderate	low	Very high	moderate	high
Lion's Bay	Moderate Exposure	Britannia copper mine	moderate	low	Extremely high	moderate	moderate
Porteau Cove		Britannia copper mine	moderate	moderate	low	low	moderate

4.1. Biomarker responses

The use of both internal and external dose biomarkers of exposure were used in this study, which allowed the detection the chemical effects of PPM and copper mine industries in the Howe Sound region. In this study, specifically MT content and EROD activity were used as specific biomarkers of contaminant (e.g. metals and AhR activating organic chemicals) exposure.

4.1.1. Metallothionein

MTs play a major role in chelating metals and scavenging free radicals, thereby preventing potential damage to tissues (McCarter & Roch, 1983). Induction of MT in aquatic organisms is an indicator of increased tolerance to heavy metals (McCarter & Roch, 1983). MT in the digestive gland of mussels have the ability to perform both hepatic and intestinal functions as well as phagocytosis and intracellular digestion thereby enabling the binding of metals to the digestive cells before being transported to other organs (Roesijadi, 1996).

In mussel digestive glands, MT levels were high from those collected at the Britannia Beach site when compared to the reference site (Chaster Bay). Britannia Beach is situated just beneath the Britannia mine, and organisms in the area were continuously exposed to a wide range of metals from the Britannia mine ore bodies. Induction of MT activity is known to be influenced by a range of stress inducing conditions (e.g. exposure to Cu and hypoxia (Costa et al., 2009). Also, the induction of MT activity may also be induced by strong synergistic inducing metals including Cd, Cu, Pb and zinc (Costa et al., 2009). Apart from the induction of MT activity by metals, it has been suggested that that oxidative stress resulting from the catabolism of organic compounds in aquatic organisms such as fish can be responsible for a significant increase in MT activity (Costa et al., 2009). Britannia Beach is approximately 7 km south of Darrell bay where the historical Wood fiber PPM was once located. The water from Darrell Bay flowed south towards Britannia Beach, Porteau Cove and Lion's Bay. The high activity of MT activity in mussel digestive glands inhabiting at Britannia beach indicates that these mussels may still be exposed to a complex mixture of trace metals and aromatic hydrocarbons when compared to the low MT activity found in Darrell Bay and Porteau Cove. Several researchers have demonstrated that MT can be induced by

exposure to both metals and organic contaminants (Costa et.al (2009). For example, Romeo et al. (1997) showed that MT activity was highly induced when fish were simultaneously exposed to Cu and benzo[a]pyrene and lower induction when exposed to these contaminants individually. Also in a study carried out by Faria et al., (2010) to investigate contaminant accumulation and multibiomarker responses, MT activity was significantly higher in organisms from sites with high PCB, Cr, Ni, C, Cd and Hg concentrations when compared to sites exposed to only aromatic hydrocarbons or heavy metals individually (Faria et al., 2010).

4.1.2. Ethoxyresorufin-O-deethylase

The results of the EROD assays showed that mussels from Britannia Beach, Porteau Cove, Lion's Bay and Port Mellon were significantly higher compared to those from Chaster Bay (reference site). The induction of CYP1A in vertebrates and invertebrates exposed to contaminants such as dioxins, furans, polychlorinated biphenyls and polycyclic aromatic hydrocarbons has been routinely used as a biomarker of exposure since CYP1A is responsible for converting 7-ethoxyresorufin-*O*-deethylase (EROD) to resorufin. Several studies have shown an induction of EROD activity in Zebra mussels exposed to different contaminants (e.g. 100 ng/l of PCB mixture of Arochlor 1260 and dioxin-like CB-126) (Binelli *et al.,* 2006). Among 17 sampling sites along an area of Italian sub-alpine great lakes, it was shown that the pollution from planar compounds were able to activate EROD activity (Binelli *et al.,* 2006). In Behrens & Segner (2005) EROD activity was measured in livers of brown trout maintained in the laboratory and in livers of brown trout exposed to urban steady flow stream. The EROD activities was sampled between October 1995 and November 1999, two trends was observed from the study: there exists a seasonal variation of hepatic EROD levels in fish from the urban steady flow stream as well as in the laboratory control, and the EROD activities of the laboratory fish are generally lower than those of the fish from the urban steady flow stream (Behrens & Segner, 2005). This is to show that the increased EROD activity observed at Porteau Cove and Lion's Bay could be attributed to urban and agricultural contaminant.

It is not always possible to have a linear concentration–response relationship between contaminants and CYP1A content and/or activity in the natural environment as there is a mixture of both inducers and inhibitors of CYP1A which are likely to act

simultaneously. Finally, other factors such as temperature, season or sexual hormones can also greatly influence the responsiveness of the CYP1A (Behrens & Segner, 2005). This is to say that the increased EROD activity observed at Howe Sound is not only directly influenced by industrial contaminants

Mussels have been extensively used to measure biological effects of contamination through measurement of their growth, physiology or reproduction. They have also been used to monitor contamination by measuring tissue accumulations by chemical analyses. The digestive gland of bay mussel or foolish mussels (*Mytilus trossulus*) were used as bioindicator to conduct both MT and EROD assay. The monitoring of trace toxic substances in aquatic environments using biological indicators has been well established, and mussels or other bivalves are commonly preferred for this purpose due to several advantages. These include their wide geographical distribution, sessile lifestyle, easy sampling, tolerance of wide range of salinities, comparatively long life-span, resistance to stress and high accumulation of a large range of chemicals, including PAH (Hellou & Law, 2003). In Hellou & Law (2003), wild mussels *Mytilus edulis* and *Mytilus trossulus* were employed to assess the tolerance to PAH as an indicator of ecosystem health.

4.2. Bioindicator responses

Bioindicators provide measures of biological responses to contaminants in the environment and are commonly used to assess integrated information about environmental contamination or effects that cannot be defined with chemical analysis. In this study; giant kelp and intertidal species diversity were used as biological indicators to assess the biological effects present at Howe Sound.

4.2.1. Giant kelp germination and tube length test

Zoospores obtained from giant kelp plant were used to test the total water column toxicity, and results obtained from the test showed a statistically significant decrease in giant kelp germination (but not germination tube length) when exposed to water from the high exposure sites (Darrell Bay, Port Mellon, Britannia Beach, and Lion's Bay). Giant kelp have potential as good indicators due to their role as primary producers to higher tropic level organism. The zoopsores of Macrocystis pyrifera, have proved to be more sensitive to contaminated water column during germination stage when compared to the tube length growth stage. In general, exposing zoospores to heavy metals have demonstrated to damage cell membranes, inhibit photosynthesis, and reduce growth rate (Chung & Brinkhuis, 1986). Burger et al., (2007), analyzed whole parts of kelp to examine the variability of metal distribution. The result obtained showed that the blades of the kelp (where the zoospores are housed) had more concentration of metals in them and were more suitable for short term exposure when assessing the effect of metals (arsenic, cadmium, chromium, manganese, lead, mercury and copper) (Burger et al., 2007).

It is believed that the sensitivity of giant kelp to chemical exposure may depends on age and developmental stage (Thelin, 1981). Although there are no detailed studies on the effects of PAH and chlorinated hydrocarbons on the germination of giant kelp, it has been speculated based on the documented effects on *Fucus serratus* (Jonsson 1997), This effect occurs due to the possibility that PAH and chlorinated hydrocarbons may disrupt germinating microscopic haploid female gametophytes initiated through a decrease in its polysaccharide mucilage. A decrease in polysaccharide mucilage may then affect the following functions; protection of roots, selectivity of ions, influence on root disease and decrease the rate of soil carbon (Oades, 1978).

4.2.2. Intertidal species richness

Intertidal species play an important role in maintaining the local ecological balance as they are located at the interface of land and water. Furthermore, the composition of sessile communities is particularly useful as a baseline for ecological monitoring because such organisms are unable to avoid disturbances in the marine environment and thus, the composition of the community reflects their common history. Intertidal species are more advantageous as bioindicator species due to their habitat as they are susceptible to both terrestrial and marine disturbances. In comparison to the sublittoral and offshore habitat, gaining access to the intertidal ecosystem is much easier and are more amenable to manage.

For this study, the intertidal community was used as a biological indicator for all selected sites. The total intertidal species available at different study sites were analyzed. Chaster Bay (reference sites) had the highest number of species followed by the moderate exposure sites and then the high exposure sites and Britannia Beach. The intertidal species observed was in accordance with the proximity of contamination source.

Although there was no statistically significant difference between the numbers of species found at different sites within Howe Sound, limpets and mussels were mostly abundant at Darrell Bay, Britannia Beach and Port Mellon. Limpets and mussels are known to be good grazers and filter feeders. Grazer have shown to establish colonization of species in disturbed environment. In a study by Gustavo et al. (2016), limpets were used to establish the colonization of divergence in an ecosystem, while filter feeders have been shown to adapt in poor water quality environment and also play a role in stabilizing and rehabilitating the ecosystem (Ostroumov, 2005).

Chapter 5. Conclusions

The use of biomarkers and bioindicators to estimate biological effect in an environment, help identify the type and the magnitude of contaminants present in an ecosystem. Specifically, this study shows the effect of pulp and paper mill and copper mine effluents on the intertidal habitat, this helps with understanding the effect of specific contaminants and its role in shaping of intertidal community.

From the results obtained, polycyclic aromatic hydrocarbons are able to induce EROD activity, EROD activity measurement on mussels proved to be good biomarkers for exposures to PAHs. The result above suggests that the use of EROD as a biomarker of exposure is a good tool for preliminary assessment for PAH, as EROD activity provides an indications that these contaminants are bioavailable at Howe Sound environment. Also it is worth noting that there factors which might limit the efficacy of

EROD and these include a variety of internal, external and temporal factors such as the size and age of the test organism, the reproductive status, temperature and pH etc. (Whyte et al., 2000).

For this project, MT assay provides us with the preliminary knowledge of how these intertidal communities are stressed within their environment and how they manage with the stressors. Also, MT serves as a stress indices for understanding the event of pollutant such as heavy metals. Although MT assay provides evidence of contaminants in an environment, MT production in mussels may not be an effective biomarker as mussels may also have other mechanisms to deal with higher levels of metals, such as glutathione enzymes.

Giant Kelp germination and tube length test was a useful bioindicator for the project as they are useful to tropic level of organism. The result obtained particular for Giant Kelp germination test showed a clear consistency with exposure rating for each study site, while the Giant Kelp tube length test was slightly the same for study sites. The difference in obtained between the germination test and the tube length may be linked to the tube length of *macrocytic pyrifera* being less sensitive when compared to the germinating zoospores.

Lastly the intertidal community played a great role in showcasing the sites that where less affected by industrial activities at Howe Sound. As shown in the table 5.1 below, an increased species richness was observed in Chaster Bay, followed by moderate exposure sites (Porteau Cove and Lion's Bay) and lastly high exposure sites (Port Mellon, Darrell Bay and Britannia Beach).

Table 5.1. List of species found at different sites across Howe Sound with common and scienttific names

Sites	Common Name	Scientific name
Chaster Bay	Alaria seaweed	*Alaria esculenta*
Chaster Bay	Amphipod grey	*Paracalliopiidae*
Chaster Bay	Anenome (pale and green center)	*Actiniaria*
Chaster Bay	Barnacle	*Cirripedia*
Chaster Bay	Black pine	*Neorhodomela larix*
Chaster Bay	Blenny fish	*Blennioidei*
Chaster Bay	Branching Coralline	*Rodophyta*
Chaster Bay	brown Bryozoan	*Bugula neritina*
Chaster Bay	Brown macroalgae	*Sargassum muticum*
Chaster Bay	Brown Sponge	*Agelas conifera*
Chaster Bay	False irish moss	*Mastocarpus Stellatus*
Chaster Bay	Fucus	*Fucus vesiculosus*
Chaster Bay	Grateloupia	*Grateloupia chiangii*
Chaster Bay	Green Seaweed	*Blidingia minima*
Chaster Bay	Green Seaweed Thick	*Blidingia marginata*
Chaster Bay	High Cockscomb	*Anoplarchus purpurescens*
Chaster Bay	Kelp Isopod	*Idotea Wosnesenskii*
Chaster Bay	Microcladia	*Microcladia borealis*
Chaster Bay	Mussel	*Mytilus edulis*
Chaster Bay	Pillbug	*Armadillidiidae*
Chaster Bay	Plate Limpet	*Lottia scutum*
Chaster Bay	Polyneura	
Chaster Bay	Porphiropsis	
Chaster Bay	Pretocladia	
Chaster Bay	Race rocks	*Castarea Costata*
Chaster Bay	Red algae	*Rhodophyta*
Chaster Bay	Red Branching Bryozoan	*Schizoporella errata*
Chaster Bay	Red Encrusted Algae	*Lithothamnion*
Chaster Bay	Ribbon Worm (purple)	*Nemertea*
Chaster Bay	Rockweed Isopod	*Pentidotea wosenesenskii*
Chaster Bay	root knot nematode	*Meloidogyne*
Chaster Bay	saddle back gunnel	*Pholis ornata*

Sites	Common Name	Scientific name
Chaster Bay	Sea Lettuce	*Ulva*
Chaster Bay	Sea moss	*Chondrus crispus*
Chaster Bay	Sea Urchin green	*Echinoidea*
Chaster Bay	Slender Cockscomb	*Anoplarchus insignis*
Chaster Bay	small red algae	*polysiphonia ceramiaeformis*
Chaster Bay	Sugar Kelp	*Saccharina latissima*
Chaster Bay	Tubeworm (Calcarious)	*Cestoda*
Porteau Cove	Barnacle	*Cirripedia*
Porteau Cove	Black Prickleback	*Xiphister atropurpureus*
Porteau Cove	Bryozoan Brown	*Bugula neritina*
Porteau Cove	Cockscomb (unknown spp)	*Anoplarchus*
Porteau Cove	Dungeness Crab	*Mtacarcinus magister*
Porteau Cove	Fucus	*Fucus vesiculosus*
Porteau Cove	Amiphipod crustacean	*Gammarus pulex*
Porteau Cove	Green Hairy Crab	*Eriocheir sinensis*
Porteau Cove	Green Seaweed	*Blidingia minima*
Porteau Cove	Green Shore Crab	*Carcinus maenas*
Porteau Cove	Gunnel (Crescent)	*Pholis laeta*
Porteau Cove	Hermit Crab	*Paguroidea*
Porteau Cove	High Cockscomb	*Pholis ornata*
Porteau Cove	Segmented worm	*Annelida*
Porteau Cove	Polysiphonia	
Porteau Cove	Purple Shore Crab	*Hemigrapsus nudus*
Porteau Cove	Red Encrusted Algae	*Lithothamnion*
Porteau Cove	Sculpin (illegible spp)	*Cottoidea*
Porteau Cove	Sea Lettuce	*Ulva*
Porteau Cove	Sea moss	*Chondrus crispus*
Lion's Bay	Barnacle	*Cirripedia*
Lion's Bay	Black Prickleback	*Xiphister atropurpureus*
Lion's Bay	Brown Bryozoan	*Bugula neritina*
Lion's Bay	Brown Sponge (aka brown bryozoan)	*Agelas conifera*
Lion's Bay	Fucus	*Fucus vesiculosus*
Lion's Bay	Amiphipod crustacean	*Gammarus pulex*
Lion's Bay	Green Seaweed thick	*Blidingia marginata*
Lion's Bay	Green Seaweed	*Blidingia minima*
Lion's Bay	Green Shore Crab	*Carcinus maenas*
Lion's Bay	Gunnel Saddleback	*Pholis ornata*
Lion's Bay	Hermit Crab	*Paguroidea*
Lion's Bay	High Cockscomb	*Pholis ornata*
Lion's Bay	Mastocarpus	*Mastocarpus stellatus*
Lion's Bay	Polysiphonia	

Sites	Common Name	Scientific name
Lion's Bay	Purple Sea Star	*Pisaster ochraceus*
Lion's Bay	Purple Shore Crab	*Hemigrapsus nudus*
Lion's Bay	Red Encrusted Algae	*Lithothamnion*
Lion's Bay	Rock Prickleback	*Xiphister mucosus*
Lion's Bay	Brown macroalgae	*Sargassum muticum*
Lion's Bay	Sea Lettuce	*Ulva*
Lion's Bay	Brittle sea star	*Ophiuroidea*
Lion's Bay	Sugar Kelp	*Saccharina latissima*
Port Mellon	Amphipod grey	*Paracalliopiidae*
Port Mellon	Barnacle	*Cirripedia*
Port Mellon	Black Prickleback	*Xiphister atropurpureus*
Port Mellon	Crescent Gunnel	*Pholis laeta*
Port Mellon	Dungeness Crab	*Mtacarcinus magister*
Port Mellon	Fucus	*Fucus vesiculosus*
Port Mellon	Green Sea Hair Thick	*Blidingia marginata*
Port Mellon	Green Seaweed	*Blidingia minima*
Port Mellon	Green Shore Crab	*Carcinus maenas*
Port Mellon	High Cockscomb	*Pholis ornata*
Port Mellon	Kelp Sugar	*Saccharina latissima*
Port Mellon	Purple Shore Crab	*Hemigrapsus nudus*
Port Mellon	Rock Prickleback	*Xiphister mucosus*
Port Mellon	Sea Lettuce	*Ulva*
Darrell Bay	Amphipod grey	*Paracalliopiidae*
Darrell Bay	Barnacle	*Cirripedia*
Darrell Bay	Fucus	*Fucus vesiculosus*
Darrell Bay	Green Seaweed	*Blidingia minima*
Darrell Bay	Isopod (unspecified)	*Idotea*
Britannia Beach	Amiphipod crustacean	*Gammarus pulex*
Britannia Beach	Barnacle	*Cirripedia*
Britannia Beach	Fucus	*Fucus vesiculosus*
Britannia Beach	Green Shore Crab	*Carcinus maenas*
Britannia Beach	Hermit Crab	*Paguroidea*
Britannia Beach	Littleneck clam	*Protothaca staminea*
Britannia Beach	Mussel	*Mytilus edulis*
Britannia Beach	Pillbug Isopod	*Armadillidiidae*
Britannia Beach	Polychaete worm (segmented worm) Nereis sp.	*Annelida*
Britannia Beach	Red Algae	*Lithothamnion*
Britannia Beach	Shield Limpet	*Lottia pelta*

5.1. Recommendations for future sampling

To sample the contamination gradient at Howe Sound, specific tests should be employed to analyze any possible contaminants present at each site to better understand their effects on the intertidal community. Additional intertidal sites would be useful, as there was a great distance between the reference sites (Chaster Bay) and the rest of the study sites; the Port Mellon site was greatly segregated from all study sites as well, and more information on the ecosystem health at Port Mellon could be better understood if additional sites around Port Mellon were selected. Rocky beaches with minimum anthropogenic disturbance and no nearby creeks or freshwater influence or beaches should be selected. Therefore, sites such as Lion's Bay and Porteau Cove could be eliminated for future studies. It would also be useful to observe and measure the following parameters: surface water salinity, pH, temperature, wave exposure and sediment load in addition to other measurements taken. Regarding the quadrat study, the number of species as well as the abundance should be recorded for each site and each site should be counted at least 5 times. Discoloration of algae or any other abnormalities should be documented as well.

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