

Effect of salmon farms on element concentrations and stable isotopes in Manila clams
and sediment in Clayoquot Sound, British Columbia

by

Nicola Rani Hannah Roberts
B. Sc., University of Victoria, 2003

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of the Requirements for the Degree of

MASTER OF SCIENCE

in the Department of Biology

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Supervisory Committee

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Dr. Asit Mazumder, Department of Biology
Supervisor

Dr. Rick Nordin, Department of Biology
Departmental Member

Dr. Jay Cullen, Department of Oceanography
Outside Member

Abstract

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Supervisor

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Departmental Member

Dr. Jay Cullen, Department of Oceanography

Outside Member

Salmon aquaculture is a controversial industry in British Columbia (BC). First Nations in BC have expressed concerns about possible contamination of traditionally harvested foods by waste from salmon farms. Trace elements are released from farms via waste feed and feces, as well as leaching from netpens and antifouling paints. In addition to elemental analysis, farm waste can also be traced using stable isotopes of nitrogen and carbon. Due to the use in salmon feed of protein and oil derived from pelagic marine fish, farm waste is typically enriched in heavier isotopes of nitrogen and carbon when compared to marine particulate organic matter. In partnership with First Nations from Ahousaht, BC, I investigated these effects by determining the concentrations of three metals and one metalloid in salmon feed, sediment and Manila clams *Venerupis philippinarum* from six sites in Clayoquot Sound, on the west coast of Vancouver Island, BC. Samples were collected from three sites near salmon farms and three reference sites in four different months spanning the traditional clam harvesting season. The results suggested that salmon feed continues to be a source of trace elements in the marine environment; however, salmon farms did not appear to be elevating concentrations in nearby clam tissue and sediment. Different environmental conditions between sites may have exerted a greater influence on elemental concentrations than farm-derived elements. Contrary to findings in earlier studies, the nitrogen signature of salmon feed was not enriched relative to marine particulate organic matter and was not a useful tracer of farm waste. This may have resulted from the reformulation of salmon feed to include greater quantities of protein and oil from terrestrial rather than marine sources. Due to the importance of Manila clams in First Nations' diets, the high density of salmon farms in the study area, the likelihood of ongoing feed reformulation, and the propensity for contaminants to accumulate over time, ongoing monitoring of sediment and bivalves in the area would be advisable.

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Chapter 1 . First Nations, salmon farms and contaminant levels in traditional foods

First Nations and traditional foods

Prior to European contact, the coastal First Nations in British Columbia (BC) enjoyed a varied and highly nutritious diet principally composed of shellfish and other invertebrates, and fish, which was supplemented with terrestrial animals, berries, root vegetables, and green vegetables (Richmond et al. 2005). With European contact, a long and dramatic decline in the use of traditional foods began. Several outbreaks of infectious diseases in the late 1700s, such as smallpox, led to massive native population losses totalling 75% or larger, and with this loss of life, a great deal of knowledge about traditional food types and methods of harvest also disappeared (Acheson 1995). Residential schools were established in the late 1800s with the purpose of assimilating aboriginal people into European cultural traditions. These operated for approximately 100 years (Wade 1995), and further reduced the capacity of native people to follow traditional lifestyles. Children were often sent away from their homes and communities which reduced the opportunities to transfer information about harvest and traditional food preparation techniques between generations, and also led to the loss of taste preferences for wild caught foods among children raised on institutional food (Hopkinson et al. 1995). And finally, since the early 1900s, the availability and access to traditional foods has been further reduced by the creation of reserves, and the reduction of harvesting territory that results from urbanization, forestry, and industrial pollution (Hopkinson et al. 1995).

First Nations now incorporate many western foods in their diet; however, the quality of these foods is often poor. In remote communities, fresh and nutritious foods are more expensive than highly processed, nutrient poor foods (Hopkinson et al. 1995). In urban areas, native diets may be poor as well, due partly to food choices (Kuhnlein 1984). The prevalence of dietary-related diseases such as adult onset diabetes is higher among First Nations than among the general population in Canada (Health Canada First Nations and Inuit Health Branch 2003) and it has been proposed that a partial reinstatement of traditional diets may help ameliorate this problem (Arnold 2003). However, the

reinstatement of traditional diets is hindered by greatly reduced access to harvest areas due to changing landscapes, fisheries legislation restricting the use of traditional food resources, and adaptation to western foods (Hopkinson et al. 1995).

In addition to these obstacles, some wild caught foods that First Nations heavily rely upon have a higher level of contaminants than store bought foods (Kuhnlein and Chan 2000). This problem is especially apparent among northern communities, where persistent organic pollutants and heavy metals are carried north via atmospheric transport. Many of these contaminants biomagnify, reaching high levels in marine fish and mammals at the top of the food chain (Fisk et al. 2003). For indigenous people in northern Canada, high rates of marine food consumption coupled with elevated contaminant levels pose serious health risks (Kuhnlein and Chan 2000; Arnold 2003).

When locally available food sources are polluted, First Nations are forced to reduce or eliminate them from their diet. In the 1970s, for example, the Dryden paper company in Ontario routinely discharged mercury into local rivers. After the detection of elevated levels of mercury in locally caught fish, and in human hair and blood samples, the northwestern Ontario Anishinabe people reduced their fish consumption by over 90% (Usher 1995). Local food sources have traditionally been an important component of First Nations diets' and the contamination of key foods seriously affects the quality of and variety in their diets.

The consumption of traditional foods is also vital to preserving native culture, and offers the additional benefits of being less expensive and more nutritious than the western foods available in remote communities (Kuhnlein and Chan 2000). First Nations must now weigh these benefits against health risks due to contaminants. In order for First Nations to make informed decisions about which foods to incorporate in their diet, contaminant levels in traditional foods must be determined and if necessary, the potential sources of pollution identified. When concerns about the health and safety of these foods arise in communities, it is important to investigate them and determine whether or not the

concern is warranted, because even the perception of contamination leads to reduced consumption of traditional foods and further erosion of First Nations cultural and physical well being (Kuhnlein and Chan 2000).

Salmon farms in BC

Since their establishment in BC in the 1970s, salmon farms have been highly controversial. The conflicting perspectives of independent researchers, government scientists, the aquaculture industry, and First Nations as well as other stakeholders, have led to highly polarized groups (Hamouda et al. 2005). Some research suggests that escapes of farmed salmon, transfers of sea lice and disease from farmed to wild salmon, and human health issues are cause for concern (Hislop and Webb 1992; Gross 1998; Krkosek et al. 2005; Weir and Grant 2005). Scientists from Fisheries and Oceans Canada refute that salmon aquaculture harms wild salmon (Beamish et al. 2006; Beamish et al. 2007; Department of Fisheries and Oceans Canada 2007) and BC salmon farmers focus on the numerous economic benefits salmon farms bring to coastal communities (BC Salmon Farmers Association 2007).

These conflicting reports result in concern and uncertainty about the impact of fish farms on traditional foods in some communities, while other communities welcome the economic benefits. The Kitasoo/ Xaixais in Klemtu have set up agreements to co-manage farms (Kitasoo Xaixais 2007), while the Musgamagw Tsawataineuk Tribal Council (MTTC) in the Broughton Archipelago struggle to reduce or eliminate them from their territories (Musgamagw Tsawataineuk Tribal Council; Schreiber 2006). First Nations from the Broughton Archipelago have reported negative changes at traditional clam beaches and are concerned that these changes could be associated with farm waste. Clams are observed to be smaller and darker in colour, and beaches that were regularly harvested are showing evidence of eutrophication (Routledge et al. 2007). Eutrophication and oxygen depletion commonly occur in sediments beneath and immediately surrounding farms when excess feed and feces are concentrated beneath net pens with low water flow (Hargrave et al. 1993; Wildish et al. 2001; Brooks et al. 2003). Whether this effect extends to nearby beaches has not been well studied.

Ahousaht is a small community of approximately 1000 people that is located on Flores Island in Clayoquot Sound on the west coast of Vancouver Island, BC (Figure 1.1). Salmon aquaculture operations have been established in Ahousaht since the late 1990s and have reluctantly been accepted by the Ahousaht community. The community benefits from employment both on farms and at the fish processing plants, which has sharply reduced the welfare rate. However, some residents are concerned about the fate of uneaten feed, salmon feces, and the contaminants that are released into the surrounding marine habitat (Stackhouse 2001).

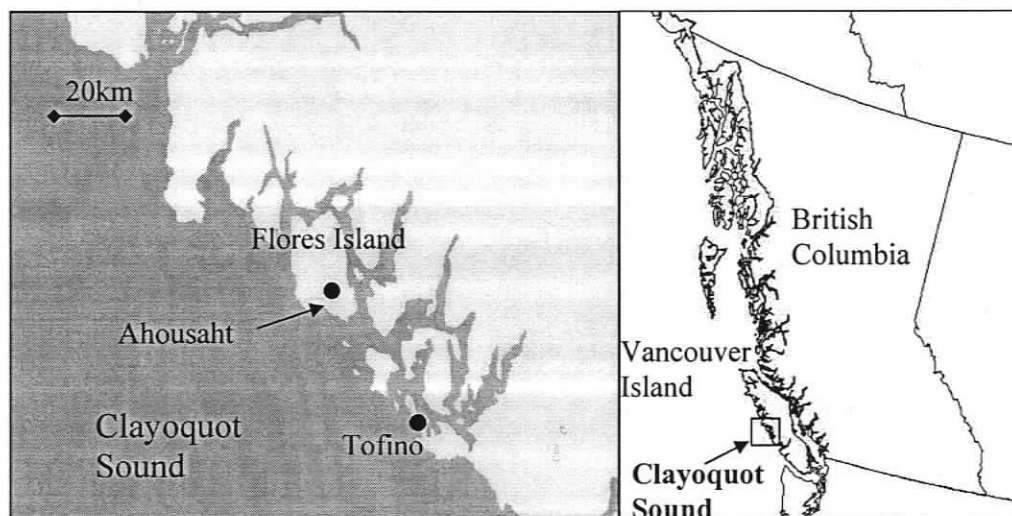


Figure 1.1. Maps depicting Ahousaht on Flores Island in Clayoquot Sound on the west coast of Vancouver Island, British Columbia, Canada.

A marine foods consumption survey conducted in 2005 found Ahousaht residents rely heavily on locally harvested seafood, and harvesters distribute their catch within the community (Marlor and Eyding 2005). Store-bought foods can be purchased in Tofino; however, this requires a two-hour round trip by water taxi and incurs travel costs. Because Ahousaht residents rely heavily on local seafood, their concerns about farm-related effects need to be investigated. If an effect of fish farms on traditional foods is not detected, hopefully residents' concerns will be assuaged and they will not reduce or eliminate these foods in their diet unnecessarily. If fish farms do have a negative effect on

foods, residents can make informed decisions about the continued presence of fish farms in their traditional harvesting territory.

Study organism

Manila clams (*Venerupis philippinarum*) are not native to BC and were first detected in 1936 (Bourne 1982). They are now widely distributed in Clayoquot Sound and by virtue of their abundance and ease of harvest they have largely replaced the native littleneck in the traditional Ahousaht diet. While not technically a traditional food, due to its non-native origins, it has filled the niche of the native species and is now one of the most widely-consumed marine foods in the community. Littleneck clams are collected incidentally while harvesting Manila clams and are still consumed by Ahousaht residents but in much smaller quantities. Manila and littleneck clams together are called “steamer clams.” More than ten tonnes of steamer clams were harvested in the Ahousaht area in 2004; many of these are consumed within Ahousaht while a large portion was also shared with family living in other nearby communities such as Tofino (Marlor and Eydling 2005).

This study examines the concentrations of metal contaminants in the soft tissue of Manila clams at sites near active fish farms and compares these to reference sites to determine whether proximity to fish farms impacts these levels. Previous studies have demonstrated that metal concentrations vary significantly with clam size and time of year (Orren et al. 1980; Saavedra et al. 2004; Ji et al. 2006), thus clams of various sizes were collected at four different times within the typical First Nations harvest season. The information collected on metal concentrations in Manila clams in Clayoquot Sound will also serve as a useful baseline if future research is undertaken to investigate the long term effects of fish farm waste (deBruyn et al. 2006).

In humans the primary route of exposure to metals is dietary, and the highest concentrations are typically found in seafood (Devesa et al. 2001; Munoz et al. 2005; Falco et al. 2006). Via the process of bioaccumulation, some elements, such as cadmium and arsenic, increase in concentration from food and water into the tissues of marine

organisms over time; this can result in high levels even when ambient concentrations are low (Griscom et al. 2002; US EPA 2003). Chronic low level cadmium and arsenic intake have been associated with adverse health effects including hypertension, increased cancer risks, and increased prevalence of diabetes (IPCS 1992; IPCS 2001).

The effects of fish farm waste on the levels of metal contaminants in nearby intertidal zones have received little attention. Using data collected from six clam beaches within the Ahousaht territory in Clayoquot Sound, I determined the concentrations of metals in the sediment and in the clam tissue near farms, and used tracers to determine whether differences between farm and reference sites were associated with farm waste.

The metal concentrations in clam tissue at sites close to farms are compared to those at reference sites in Chapter 2. I have accounted for the natural variability that is associated with the time of year the clams were collected and clam size (shell length). I also compared the sediment metal concentrations at farm and reference sites and looked for correlations between sediment and tissue metal levels. I used geonormalization techniques to distinguish farm effects from natural variability in sediment metal concentration.

In Chapter 3, I examined the utility of stable isotopes of carbon and nitrogen to trace farm-derived waste in sediment and clams at sites near salmon farms. In Chapter 4, I concluded with a general discussion of the research presented in this thesis as well as future research directions.

Chapter 2 . Metal concentrations in Manila clams and sediment near salmon farms in Clayoquot Sound, BC

Abstract

Along with feed waste and fecal material, metal and metalloid compounds (collectively referred to as metals for simplicity) associated with salmon aquaculture are released into the marine environment. Farm siting criteria prohibit situating farms within 300m of shellfish beds that are harvested by First Nations; however, if waste travels further, this criterium may not provide adequate protection. The Ahousaht First Nations live in Clayoquot Sound, off the west coast of Vancouver Island, British Columbia (BC), and Manila clams are a staple in their diet. An influx of farm-derived metals to nearby shellfish beds in Ahousaht harvesting territory could elevate concentrations in clams due to an increase in ambient levels of metals. Conversely, waste from farms could increase ambient nutrient levels and lead to higher growth rates in clams at farm sites and reduced metal concentrations due to the process of biodilution, which occurs when tissue growth is more rapid than the rate of metal uptake. To test these hypotheses, sediment and Manila clams were collected from three shellfish beds between 500 and 700m from fish farms and from three reference sites 1 to 10km from active farms. The metal concentrations and tissue weights, as well as the variability between sampling months, were compared in the sediment and Manila clams from farm sites to those at reference sites. The ratios of both copper and zinc to lithium were also analysed to distinguish natural variability in the metal concentrations in sediment from aquaculture-derived effects. The results suggest that differences between farm and reference sites in the arsenic, cadmium, and zinc concentrations in clams were due to values at two reference sites and were not attributable to farm proximity. These two sites differed from the other four sites in that they were situated at the end of narrow channels. Clams from all reference sites had marginally less tissue mass than clams from farm sites which suggested that farm waste may provide some nutrients without being a significant source of metals. Sediment at farm sites did not have higher metal concentrations than sediment from reference sites and no differences were detected between farm and reference sites in the variability of metal concentrations in either the sediment or bivalves. The ratios of

copper and zinc to lithium overlapped at farm and reference sites and indicated that the concentrations of metals in the sediment at farm sites were similar to background levels. Differences in metal concentrations in clams and sediment were generally site-specific. Ideally, future studies would determine baseline metal concentrations in clams and sediment before salmon farms are established and monitor changes over time to eliminate the difficulty in finding properly matched farm and reference sites.

Introduction

There are several chemicals used in aquaculture that may subsequently be introduced into the marine environment (Burrige 2003). These include intentionally used chemotherapeutants and antibiotics (Jacobsen and Berglund 1988; Ervik et al. 1994; Weston 1996; Burrige 2003), persistent organic pollutants which are concentrated in the fish oil component of salmon feed (Jacobs et al. 2002; Karl et al. 2002; Hites et al. 2004; Berntssen et al. 2005; Hellou et al. 2005), and heavy metals, such as zinc and copper, that are associated with cage structures and antifouling paints (Zitko 1994; Chou et al. 2002). Zinc and copper are also added to feed as nutritional supplements (Berntssen et al. 1999; Vangen and Hemre 2003), while the metalloid arsenic, and the metal cadmium (collectively referred to as metals in this document for simplicity) may be present incidentally in the fish by-products used in feed (Sutherland et al. 2001; Chou 2007; Dean et al. 2007).

In the Bay of Fundy, Burrige et al. (1999) found that the concentration of copper and zinc in sediment collected from 25 to 100m from salmon net pens exceeded the CCME marine interim sediment quality guidelines (ISQG) of 18.7ppm and 124ppm, respectively (Canadian Council of Ministers of the Environment 2002). Also in the Bay of Fundy, Smith et al. (2005) found that copper and zinc concentrations in the sediment were elevated above background levels at distances of greater than 200m from salmon cages. Similar findings have been reported from Scotland, where Dean et al. (2007) found elevated levels of copper and zinc in sediment at ~300m from fish farms, and of cadmium at distances of ~100m from fish farms.

According to mass balance calculations by Cross (2005), approximately 35% of zinc administered to net pens in the form of fish feed is lost to the environment.

Approximately half of the waste released from salmon farms was estimated to become available for downstream transport in dissolved form or in suspension, while the remainder was estimated to settle beneath the pens. Near Sonora Island, British Columbia (BC), Cross (2005) found zinc and cadmium were elevated in sediment at distances from 75 to 200m downstream of farms. Whether this becomes available to nearby intertidal organisms is not well known.

Changes to copper and zinc concentrations do not necessarily follow a simple concentration gradient, making it difficult to discern changes associated with salmon farms from variability in the sediment concentrations that are due to granulometry, mineralogy, and organic content (Sutherland et al. 2007). To distinguish anthropogenic effects from the natural variability in sediment metal concentrations, Loring (1990) proposed normalizing copper and zinc to lithium concentrations. This method controls for the effects of grain size and shows less variability between reference sites than normalising metal concentrations to organic carbon (Chou et al. 2002). Sutherland et al. (2007) successfully used this approach to detect aquaculture derived changes in copper and zinc concentrations in sediment at farm sites.

Recently researchers have begun to investigate whether metals associated with salmon aquaculture are also transferred to the fauna living close to farms. In Passamaquoddy Bay, New Brunswick, lobsters living within 50m of farm sites had elevated levels of copper (Chou et al. 2002) and sea urchins within 75m of sites had elevated levels of copper and zinc (Chou et al. 2003). Cross (2005) found tissue zinc concentrations in scallops situated from 40 to 150m of salmon cages were elevated relative to scallops found beyond 150m.

While a few studies have demonstrated that the tissues of organisms living close to farms have elevated levels of metals (Chou et al. 2002; Chou et al. 2003), even less is

known about metal concentrations in organisms living further from salmon farms. In one of the only studies examining concentrations of metals in organisms situated further afield, deBruyn et al. (2006) determined that rockfish living 250 to 750m downstream of farms had elevated mercury levels relative to rockfish from reference sites. Although the concentration of mercury in farm feed and farm waste is generally low (Choi and Cech 1998; Chou 2007), changes at the ecosystem level, such as increased mercury methylation by the bacteria inhabiting anoxic sediment beneath net-pens, and an elevated trophic position in the fish living close to farms, can result in higher mercury concentrations (deBruyn et al. 2006).

The size of the area over which farm waste is dispersed depends on the surface area of the farm, the depth of the water, the current velocity, and the settling velocity of waste feed and food particles (Gowen and Bradbury 1987). Holmer (1991) determined that waste products from fish farms may disperse in the surrounding marine environment in an area equivalent to ten times the size of the farm from which they originate. In BC, net-pen systems frequently consist of six pens of 20m diameter arranged in two rows (Sutherland et al. 2001). Accordingly, farm waste could disperse up to 1.2km from the source.

To protect aboriginal clam harvesters from these contaminants, the Ministry of Agriculture and Lands stipulates that aquaculture facilities cannot be sited within 300m from intertidal shellfish beds that are regularly or traditionally used by First Nations and that would be exposed to water flow from salmon farms (BC Ministry of Agriculture and Lands 2000). If waste travels further than 300m from salmon farms and is consumed by bivalves harvested by First Nations, this regulation will not provide adequate protection.

Over the 18 to 24 month period encompassing the production cycle, salmon farms continually release arsenic, cadmium, copper and zinc contained in waste feed and feces, as well as via leaching from the salmon cage structures. Copper is also released sporadically when antifouling paints are applied to net pens (Cross 2005). Therefore,

metal concentrations in clam food and water may be higher at farm sites, resulting in increased concentrations in clam tissue. Furthermore, clams distant from farms must rely mostly on phytoplankton and picocyanobacteria, which fluctuate in abundance over the year. In periods of low food abundance, such as over-winter, the metal concentrations in bivalves from reference sites might increase as rates of excretion and metabolism are reduced (Bryan 1973).

If growth rates are enhanced at farm sites, it is also possible that clams at farm sites could have lower tissue metal concentrations than clams from reference sites due to the process of biodilution, which occurs when tissue growth is more rapid than the rate of metal uptake (Leung and Furness 1999; Leung and Furness 2001). Growth dilution occurs in farmed salmon, for example, which have lower metal biomagnification factors than wild salmon due to rapid growth resulting from the consumption of nutrient dense salmon feed (Kelly et al. 2008). Researchers have found that salmon feed and feces can be a major food source to clams (Shpigel and Fridman 1990; Shpigel and Blaylock 1991; Neori and Shpigel 1999). Stirling and Okumus (1995) hypothesized that mussels grown near salmon farms in Scotland used organic waste from farms to supplement their diets. A study in Norway found mussels growing on farms grew twice as fast as those growing further from farms and attributed this to the steady year round supply of food at farms; mussels from reference sites had growth-stoppage rings which coincided with periods of low winter food availability while mussels from farm sites did not (Wallace 1980). Metal concentrations in clams growing near farms may also be more stable throughout the year than in clams from reference sites due to the consistent source of food.

Farm effects need to be distinguished from changes in metal concentrations in bivalve that are associated with clam growth. The concentration of non-essential metals, such as cadmium and arsenic, increase as clams grow due to a reduction in the rates of elimination coupled with non-regulatory uptake (Usero et al. 1997; Ji et al. 2006). Essential metals, however, such as copper and zinc, do not usually show an increase in concentration with size or time because they are regulated (Rainbow and Phillips 1993).

Several other factors may influence metal concentrations in clams and confound the effect of farms: tissue metal concentrations fluctuate seasonally due to an increase in tissue mass associated with spawning and a subsequent dilution of metals (Ji et al. 2006); upwelling may bring metals that are usually found in greater concentrations in deeper waters, such as cadmium and zinc, to the surface from where they may be transported to intertidal zones and ingested by bivalves (Lares and Orians 1997; Lares et al. 2002); and increased rainfall can lead to fluctuations in ambient intertidal metal concentrations due to the run-off of terrestrial particulate metals (Paez-Osuna et al. 1995).

To determine whether the intertidal shellfish beds that are harvested by First Nations are receiving farm-associated metals derived from waste, six intertidal shellfish beds in traditional Ahousaht harvesting territory were selected. Three of these were between 500 and 700m from fish farms and three were reference sites situated 1 to 10km away from active farms. Manila clams *Venerupis philippinarum* were actively harvested at all of the study sites by Ahousaht First Nations (Marlor and Eyding 2005) and were chosen as the study organism for this research because they have a demonstrated ability to act as biomonitors of metal contaminants (Ji et al. 2006). They respond to the biologically available metal fraction in the environment, are sedentary, abundant, and easy to sample and identify (Rainbow and Phillips 1993; Boening 1999). Manila clams feed on waste from fish farms and have been reared successfully in fish farm effluent (Shpigel and Fridman 1990; Mazzola and Sara 2001). They are selective suspension feeders (Kasai et al. 2004) and typically consume phytoplankton and seston. Resuspended sediment can also be a major food source and sediment-bound metals are assimilated by Manila clams (Chong and Wang 2000).

Coastal First Nations consume more seafood than the average Canadian and face greater risks from seafood-borne metal contamination. Barring occupational exposure, chronic exposure to low levels of non-essential metals, such as arsenic and cadmium, primarily occurs by consumption of seafood (Devesa et al. 2001). Elevated arsenic and

cadmium levels have been associated with adverse health effects such as early on-set diabetic renal complications and hypertension (Satarug et al. 2004; Navas-Acien et al. 2008) and these disorders are more prevalent among First Nations in Canada than the national average (Health Canada First Nations and Inuit Health Branch 2003).

The study objectives are to:

- 1) determine whether farm waste is affecting the metal concentrations in Manila clam tissue and sediment at farm sites;
- 2) assess whether metal concentrations are less variable at farm sites than at reference sites throughout the sampling period;
- 3) compare whether clams from farm sites have greater tissue weight than the clams from reference sites to determine whether biodilution could be occurring, and;
- 4) distinguish the natural variability in sediment metal concentrations from aquaculture-derived effects using ratios of both copper and zinc to lithium as a tracer.

Methods

Field Methods

Manila clams were collected in four different sampling periods (April 25 to 27, October 17 to 19, and December 1 to 4, 2005, and February 27 to March 1, 2006) from six sites in Clayoquot Sound, BC (Fig. 2.1). Three sites were from 500 to 700m from salmon farms (near farm sites) and three were 1 to 10km from farm sites (reference sites); sites were chosen in partnership with Ahousaht First Nations and selection depended on local knowledge of currents and tidal flows. The reference site at Atleos Beach (Figure 2.1) was only slightly further away from a salmon farm than the farm sites; however, it was judged to be hydrologically distinct as it was across the channel from the farm. Clam growth rates vary with length of immersion (i.e., food availability) (Gillmor 1982) and faster-growing clams can have lower tissue metal concentrations due to biodilution (Leung and Furness 1999; Leung and Furness 2001). To reduce this effect, clams were always collected from the lowest point on the beach at the lowest tide of the month,

which ranged from 0.7 to 2.3 feet over the entire study period. Two sites were sampled each day; one just prior to and one post-low tide.

Clams were collected using clam rakes and forks, which were thoroughly rinsed between each site. Because tissue metal concentration is affected by reproductive status (Paez-Osuna et al. 1995; Ji et al. 2006), only clams large enough to have reached reproductive maturity were collected (Holland and Chew 1974). Clams were not collected within 3m of fresh water streams. The clams were measured using Vernier calipers and sorted into seven size classes based on shell length: 25, 29, 34, 39, 43, 48, and 52 ± 1.5 mm. The clams were rinsed in seawater, wrapped in Fisher aluminum foil, double bagged in Ziploc bags, and frozen at -20°C . The top 2cm of beach sediment was also collected in triplicate from each beach near the site of clam collection using a stainless steel scoop and placed in new borosilicate glass jars.

Three feed samples were collected from Clayoquot Sound; one from Millar Channel, one from Dixon Bay and one from Herbert Inlet. These farms, as well as the farm at Ross Pass, were operated by Mainstream, and the assumption was made that all of the farms under the same management use the same feed. Feed was stored in coolers in new borosilicate glass jars while in the field, then frozen at -20°C until analysed. Two Skretting salmon feed samples were also collected from the Aquatics Centre at the University of Victoria (Appendix A).

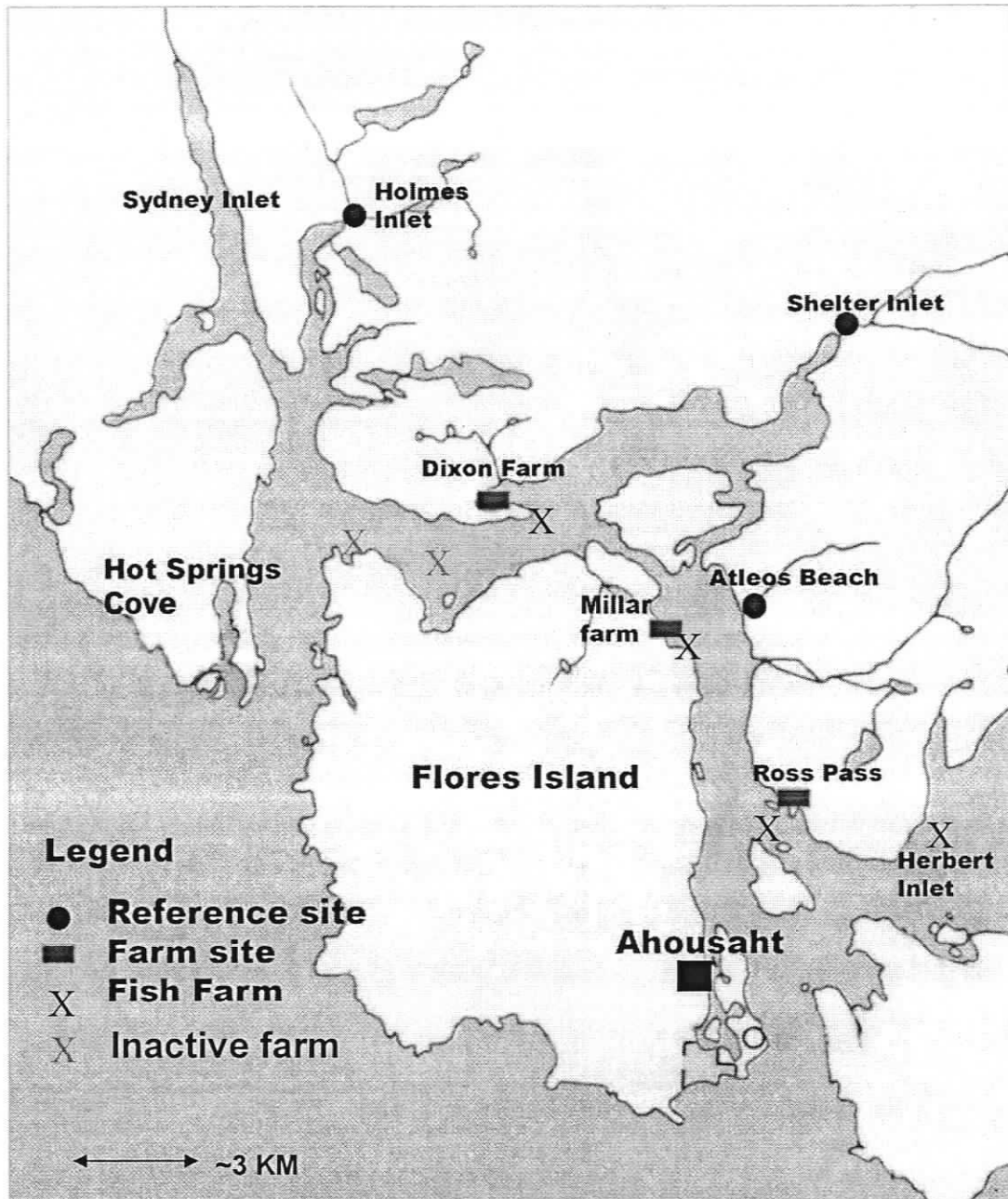


Figure 2.1. Map depicting six intertidal study sites in Clayoquot Sound on the west coast of Vancouver Island, BC. Three sites were near salmon farms and three were reference sites.

Laboratory Methods

Clams were defrosted overnight in the fridge and thoroughly rinsed in deionized water to remove any traces of sediment retained in the gills. The soft tissue for all clams in each size class was removed using a new surgical stainless steel blade, then pooled and homogenized in a stainless steel blender for 30 to 40 seconds. Two 5g sub-samples were retained for metal analysis so that every tenth sample could be analysed as a blind duplicate.

Sediment samples were freeze dried for 48 hours in a Labconco Freezone 12L Freeze Dry System (Kansas City, MI) and then gently crushed. Metal assimilation in Manila clams is not known to be affected by sediment particle size (Fan and Wang 2001), however, sediment was sieved through a 200 μ m sieve to obtain the fine fraction which better represents food particles of the size Manila clams ingest. Although a smaller size fraction was preferable (Fan and Wang 2001), the quantities of sediment were insufficient for analyses in a smaller size range.

Feed, sediment and frozen tissue samples were shipped to Maxxam Analytics (Burnaby, BC, Canada) for arsenic (As), cadmium (Cd), copper (Cu), and zinc (Zn) analyses. Samples were analyzed using inductively coupled plasma mass spectrometry on a Perkin-Elmer Elan 6000 ICP-MS. Replicability was within 10% or better for certified reference materials and recovery was typically 90 to 110% (maximum 122%). Ten mL of acid mixture (with 25% HCl and 25% HNO₃, 50% DI water) was added to approximately 2g wet weight of homogenized tissue or dry weight of sediment and digested at 90-95°C for 1 hour in a water bath (5g of sample was used for tissue with low reporting detection limits). This mixture was then diluted to 50mL with deionized water. Each batch of 20 samples included the following quality assurance/quality control samples: One blank, one blank spike, one duplicate, one reference sample, and two blind duplicates.

Data analyses

Analysis of covariance (ANCOVA) was used to test for differences in tissue metal concentrations and tissue weight between farm and reference sites, over multiple seasons, among clams of different lengths. Analysis of variance (ANOVA) was used to test for differences in metal concentrations in sediment between sites over multiple seasons. Residuals were visually inspected to confirm that normality and homogeneity of variance assumptions were not violated and data were transformed when necessary. All statistical procedures were performed using SPSS 15.0 software and in all cases, $p < 0.05$ was considered significant.

Differences between farm and reference sites in the variability between months in the metal concentrations and tissue weights in clams were compared using regression analysis. Regressions were plotted of the metal concentrations against the shell length and of the tissue weight against the shell length and ANOVA was used to compare the unexplained residual variance between farm and reference sites. To compare the variability in the metal concentrations in sediment between farm and reference sites, the coefficient of variation was determined at each site and compared using ANOVA with farm as a categorical factor.

ANCOVA was performed with either the copper or zinc sediment concentration as the dependent variable, the lithium concentration as the covariate, and farm and reference sites as categorical factors. Regressions were then plotted to examine the differences between farm and reference sites in the ratios of the metal concentrations in sediment (Sutherland et al. 2007).

Results

To determine whether farm waste could be affecting the metal concentrations in Manila clams, I start by presenting the metal concentrations in salmon feed and then addressing the differences in metal concentrations in tissue between farm and reference sites. Then the tissue weights at a given shell length are compared in clams from farm sites to clams from reference sites to determine whether biodilution could have occurred. Next, the

metal concentrations in sediment are compared between farm and reference sites to determine whether there were higher concentrations of metals in the sediment at farm sites. Finally, the ratios of both copper and zinc to lithium are used to distinguish natural variability in metal concentrations in sediment from aquaculture-derived effects.

Sources of variation in the metal concentration and tissue weight of clams

The mean concentrations of arsenic, cadmium, copper, and zinc in salmon feed in this study were within the same range as values detected by Kelly et al. (2008) and Petersen et al. (2005) (Table 2.1). BC produced over 70 000 tonnes of salmon in 2006 (Department of Fisheries and Oceans Canada 2009) and assuming that 15% of the salmon feed was lost to the marine environment (Cross 2005), 82 256 tonnes of feed were applied and 12 256 tonnes were lost.

The quantity of copper and zinc required by salmon is not well known so they are added in excess of the estimated requirements (Dean et al. 2007). Cross (2005) estimated that a greater percentage of elements than total feed are lost, although the exact quantities depend on the ability of the fish to regulate absorption, as well as on the total concentration in the water. According to Cross (2005), approximately 35% of zinc administered to netpens in the form of feed is released into the marine environment. This represented 3.4 tonnes of zinc from salmon feed and feces province-wide in 2006, as well as an unknown amount that leached from cage structures. This percentage is assumed to be similar for other micronutrients such as copper, so I estimated that 675kg of copper was released in 2006, as well as an additional unknown quantity from the periodic application of copper-based antifouling paints. The majority of antifouling paints used on salmon farms contain copper and these are a greater source of copper than salmon feed and feces (Dean et al. 2007). The percentage of metals Cross (2005) estimated are lost to the environment cannot be readily applied to arsenic and cadmium, which are not essential nutrients and follow different bioaccumulation patterns (Boyden 1974; Luoma and Rainbow 2005).

The zinc concentrations were higher and the arsenic and cadmium concentrations were lower in clams collected from farm sites (Figure 2.2), although the differences in arsenic and cadmium depended on the month and length of the clam, respectively (Table 2.2 and Appendix B). The differences between farm and reference sites in metal concentrations were driven by the values in the clams from Holmes Inlet and Shelter Inlet (Figure 2.2). There were no significant differences in the copper concentrations in clams between farm and reference sites (Table 2.2).

There was a strong correlation between all four of the metal concentrations and the shell length (Table 2.2). The arsenic and cadmium concentration increased significantly with the shell length while the copper and zinc concentration decreased (Table 2.3). The cadmium and arsenic concentrations were 1.5 and three times as high in the largest clams compared to the smallest clams, while the zinc and copper concentrations were on average 14% lower in the largest clams (Figure 2.2). The relationship between the copper or zinc concentration and the shell length was not consistent in all months that the clams were collected; in some months at some sites the copper and zinc were not correlated with shell length, whereas in other months copper and zinc increased with shell length (Appendix B). The months in which clams had the lowest metal concentrations in tissue also varied, both with the site, the length of the clam, and the metal examined. For example, the month in which the arsenic, copper and zinc concentrations were the lowest at Ross Pass depended on the size of the clam, while the cadmium concentration was the lowest in all size classes in December (Appendix B). These patterns were different between the sites.

Clams from farm sites had significantly greater tissue weights than clams from reference sites (Table 2.4), although this effect was consistent neither between sites nor between months (Figure 2.3). December was the only month in which clams from all the farm sites were heavier than clams from the reference sites, and this was only true for the larger clams (Appendix C).

Clams from two of the reference sites, Holmes Inlet and Shelter Inlet, had higher arsenic and cadmium concentrations than clams from the other sites (Figure 2.2). The slope of the regression of the arsenic concentration in tissue against the shell length was also steeper at Holmes Inlet and Shelter Inlet, indicating that at these sites the larger clams accumulated arsenic at a faster rate than at the other sites (Figure 2.2). In most of the months sampled (October, December, and February), larger clams from Holmes Inlet and Shelter Inlet also had lower tissue weights than clams from the other sites (Figure 2.3), suggesting that the large clams at these sites acquired tissue mass more slowly than at the other sites.

The magnitude of the variability in tissue weight and tissue arsenic, cadmium, copper, and zinc concentrations did not differ significantly between farm and reference sites (ANOVA; $p > 0.05$ for all comparisons). Variability at farm and reference sites overlapped for all comparisons except for the arsenic concentrations in tissue, where the unexplained residual variance at farm sites ranged from 12 to 13%, and at reference sites from 16 to 23%, although the difference was not significant. Unexplained residual variance averaged 25% for most comparisons, with the exception of tissue cadmium and zinc concentrations, which averaged 61 and 82%, respectively.

The metal concentrations in Manila clam tissue fell within the range of those detected in Manila clams from other areas in Canada, as well as in Korea, China and Spain (Table 2.5). The concentrations overlapped those detected by the Canadian Food Inspection Agency (CFIA), with the exception of cadmium, which was detected at levels almost twice as high as those found by the CFIA. The arsenic and cadmium levels in feed overlapped the mean concentrations in Manila clams, while the copper and zinc concentrations were higher in feed than in clams (Table 2.2 and 2.5).

Table 2.1. A comparison of the mean metal concentrations (mg/kg wet weight) in salmon feed from this study to the concentrations found in other studies in BC.

<i>Metal</i>	<i>This study (95% CI)</i>	<i>Kelly et al. (2008) (95% CI)</i>	<i>Petersen et al. (2005) (min. and max.)</i>
As	1.43 (0.36-2.5)	2.1 (0.82-5.3)	0
Cd	0.28 (0.07-0.49)	0.43 (0.15-1.2)	0.39 (0.3-0.48)
Cu	8.2 (2.0-14.5)	13.1 (5.5-30.8)	8.6 (7.5-10.4)
Zn	119.2 (13-225)	169 (62-458)	98.3 (78-137)

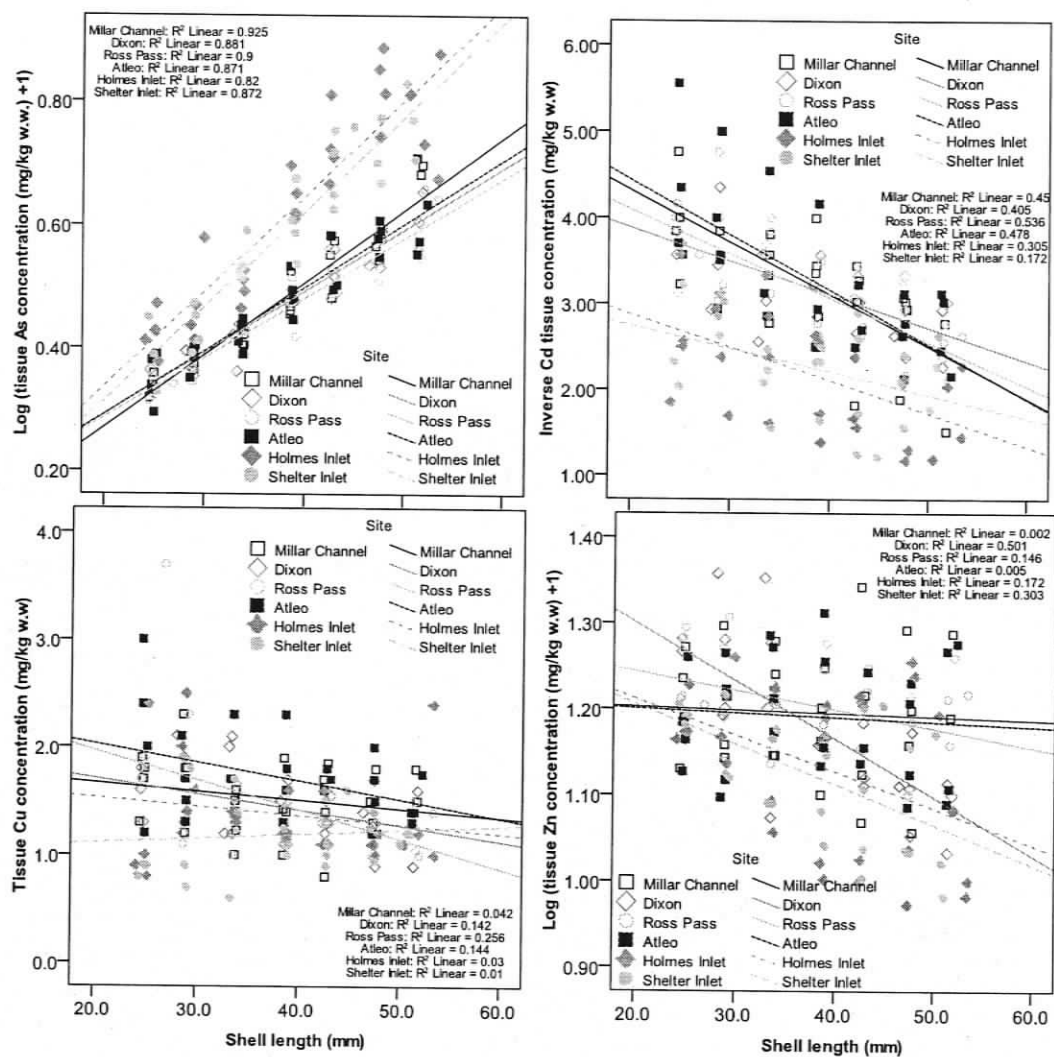


Figure 2.2. Regressions of the tissue arsenic, cadmium, copper, and zinc concentrations (mg/kg wet weight) in Manila clams against the shell length. Clams were collected in April, October, December, and February, and data from all four sampling months are combined. Open symbols are farm sites and filled symbols are reference sites. Note- cadmium concentrations are in the reverse rank order to the original data.

Table 2.2. Evaluation of the differences in the metal concentration in Manila clams (n=163; seven size classes ranging from 25 to 53mm \pm 1.5) between three farm and three reference sites in four different months using ANCOVA. Residuals were visually inspected to confirm that normality and homogeneity of variance assumptions had not been violated. The log of arsenic and zinc concentrations and the inverse of cadmium concentration were used to correct for heteroscedasticity. The dependent variable is metal concentration (mg/kg w.w.), season is a random factor, and shell length is a covariant.

Metal	Source	Df	F	Sig.	Observed Power
Arsenic	Farm treatment	1	0.93	0.337	0.16
	Month	3	4.31	0.006	0.86
	Shell length (mm)	1	491.46	<0.001	1.00
	Farm * shell length (mm)	1	10.28	0.002	0.89
	Error	156			
Cadmium	Farm treatment	1	6.79	0.080	0.43
	Month	3	1.57	0.361	0.16
	Shell length (mm)	1	98.25	<0.001	1.00
	Farm * Month	3	7.83	<0.001	0.99
	Error	156			
Copper	Farm treatment	1	3.47	0.064	0.46
	Month	3	7.04	<0.001	0.98
	Shell length (mm)	1	14.35	<0.001	0.96
	Farm * shell length (mm)	1	3.02	0.084	0.41
	Error	156			
Zinc	Farm treatment	1	29.54	<0.001	1.00
	Month	3	62.54	<0.001	1.00
	Shell length (mm)	1	51.85	<0.001	1.00
	Error	156			

Table 2.3. The relationship between the shell length (mm) and the metal concentration (mg/kg wet weight) in Manila clams collected from six study sites in Clayoquot Sound, BC, between April, 2005 and February, 2006.

Metal	Y-intercept (a)	Regression coefficient (b)	Standard error of b	r^2	Significance in regression (p)
As ^a	0.057	0.012	0.001	0.651	< 0.001
Cd ^a	4.723	-0.048	0.001	0.270	< 0.001
Cu	1.956	-0.013	0.004	0.073	< 0.001
Zn ^a	17.885	-0.100	0.023	0.105	< 0.001

The regression equation is $Y=bX + a$, where X is the mean shell length for each composite of a single size class (n=163; seven size classes ranging from 25 to 53mm+/- 1.5); Y is metal concentration; r^2 is the coefficient of determination.

^aTo correct for non-homogeneity of variance, the arsenic and zinc concentration were log transformed and the inverse of the cadmium concentration was used.

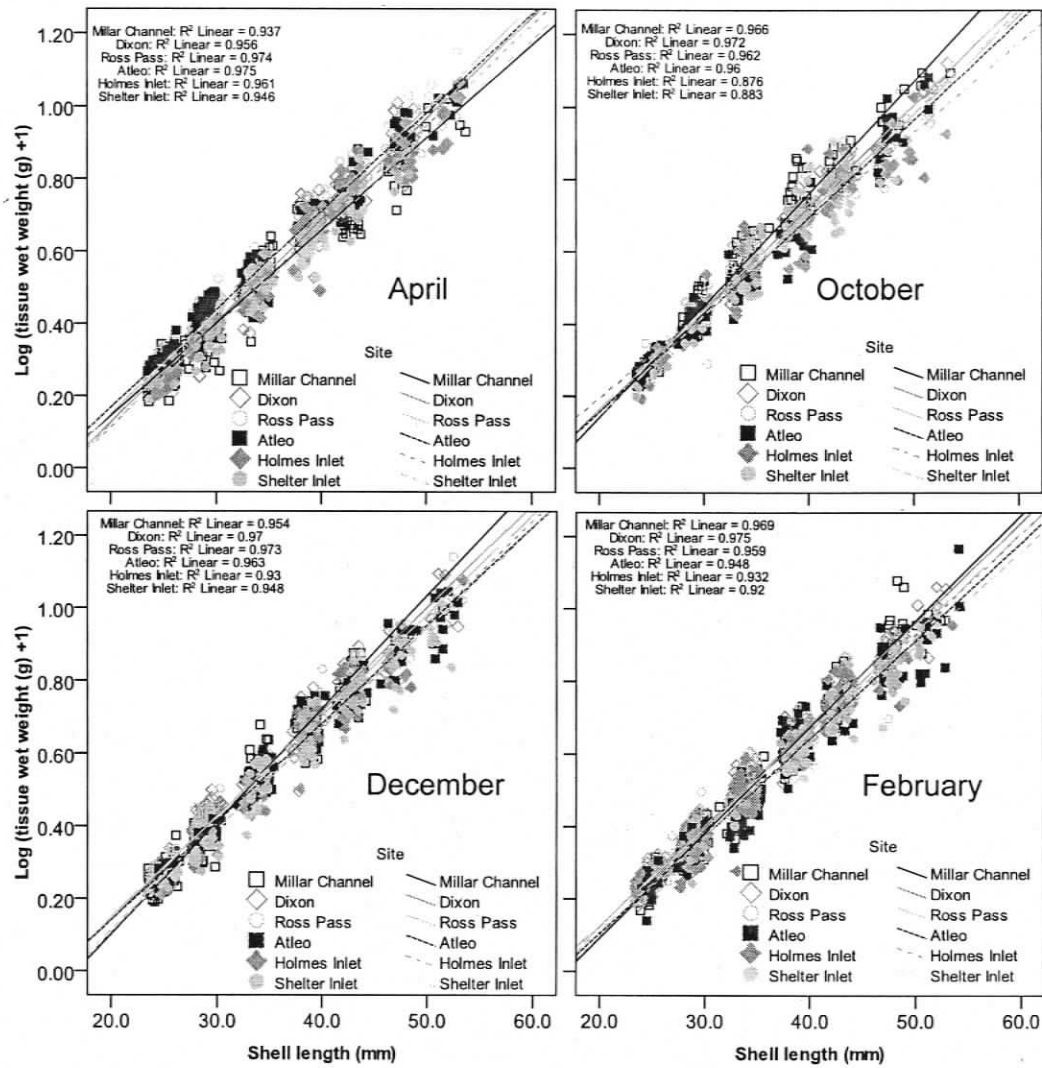


Figure 2.3. The relationship between the tissue weight and the shell length of Manila clams at farm and reference sites in four different sampling months. Open symbols are farm sites and filled symbols are reference sites.

Table 2.4. Results from a comparison of Manila clam tissue weight between farm and reference sites using ANCOVA. Clams were collected in four months (April, October, December, and February). Month was considered a random factor and shell length a covariate. Residuals were visually inspected to confirm that normality and homogeneity of variance assumptions had not been violated.

	<i>df</i>	<i>F</i>	<i>Sig.</i>	<i>Observed Power</i>
Farm treatment	1	8.5	0.004	0.83
Month	3	4.4	0.005	0.87
Shell length (mm)	1	2099.7	<0.001	1.00
Error	157			

Table 2.5. A comparison of the minimum and maximum metal concentrations (mg/kg wet weight) in the tissue of Manila clams (n=163; seven size classes ranging from 25 to 53mm +/-1.5; tissue wet weight from 0.7 to 11.1g) from beaches in Clayoquot Sound, BC, to the concentrations found in other studies.

Metal	This study	CFIA ^a	(Ji et al. 2006) ^b	(Li et al. 2006) ^b	(Liang et al. 2004)	(Usero et al. 1997)
As	0.97- 6.70	1.18-9.55				1.5- 5.2
Cd	0.18- 0.85	0.14-0.44	0.09- 0.37	0.09- 0.11	0.14- 0.63	0.06- 0.42
Cu	0.6- 3.7	0.4-2.8	0.9- 2.4	1.09- 3.36	1.3- 4.4	1.6- 3.7
Zn	8.4- 21.8	5.6-28.9	11.0- 27.5	6.0- 14.5	10.0- 20.1	12- 24

^a Metal concentrations from the Canadian Food Inspection Agency (CFIA) are a compilation of data recently collected from various unspecified locations in Canada and may represent both pristine and polluted areas.

^b Metal concentrations that were based on dry tissue weight from other studies were converted to data based on wet tissue weight by assuming 83% water content (Usero et al. 1997).

Sources of variation in the metal concentration in sediment

Significant differences in the arsenic, cadmium, and zinc concentrations in sediment were detected between farm and reference sites (Table 2.6). The mean concentrations of all four metals were lower in the sediment from farm sites but these differences were driven by the low values at the Dixon farm site and the high values at the reference site at Holmes Inlet (Table 2.7). The concentrations were generally lower in the sediment collected at Dixon, Ross Pass and Shelter Inlet than at the other sites (Table 2.7) and there were few differences between months at these three sites (Appendix D). The metal

concentrations in the sediment at Millar Channel and Atleos Beach were similar (Table 2.7) and for each of the metals investigated the variation between months was greater at these two sites than at the other sites (Appendix D). While the concentrations of all four metals in the clams were similar at Shelter Inlet and Holmes Inlet, the concentrations in the sediment did not follow the same patterns. No significant differences were detected between farm and reference sites in the variability of any of the metal concentrations in the sediment (ANOVA; $p > 0.05$ for all comparisons).

Table 2.6. A comparison of the metal concentrations (mg/kg dry weight) in sediment samples (n=70) collected from three sites near salmon farms and three reference sites in April, October, and December, 2005, and February, 2006 using ANOVA. Residuals were visually inspected to confirm that normality and homogeneity of variance assumptions had not been violated. To correct for heteroscedasticity, the arsenic and zinc concentration were log transformed and the inverse of the copper concentration was used. The dependent variable is the metal concentration and season is a random factor.

Metal	Source	df	F	Sig.	Observed Power
Arsenic	Farm treatment	1	15.35	<0.001	0.97
	Month	3	0.41	0.748	0.13
	Error	65			
Cadmium	Farm treatment	1	14.00	<0.001	0.96
	Month	3	0.36	0.781	0.12
	Error	65			
Copper	Farm treatment	5	1.73	0.193	0.25
	Month	3	0.38	0.758	0.12
	Error	65			
Zinc	Farm treatment	1	35.01	<0.001	1.00
	Month	3	0.78	0.511	0.21
	Error	65			

Table 2.7. The mean metal concentrations in the sediment (mg/kg dry weight) at each site followed by the standard deviation. ANOVA followed by Tukey HSD post hoc tests were used to identify significant differences between sites unless the variances were unequal, in which case Dunnett's T3 test was used instead. Homogenous subsets are indicated with alphabetical superscript.

Site	Metal concentration (mg/kg dry weight)			
	Arsenic	Cadmium	Copper	Zinc
Millar Channel	5.1 (1.1) ^a	0.15 (0.1) ^{ab}	29.0 (10.8) ^{ac}	46 (7) ^a
Dixon	2.0 (0.5) ^b	0.09 (0.1) ^a	11.5 (1.2) ^b	28 (2) ^b
Ross Pass	2.8 (0.7) ^b	0.10 (<0.1) ^a	22.5 (3.1) ^a	30 (3) ^b
Farm (mean)	3.2 (1.5)	0.11 (0.6)	21.0 (9.6)	34 (9)
Atleos	4.5 (1.4) ^a	0.18 (0.1) ^b	34.6 (10.2) ^c	44 (10) ^a
Holmes Inlet	8.6 (1.0) ^c	0.25 (0.1) ^c	33.9 (3.0) ^c	68 (4) ^c
Shelter Inlet	2.9 (1.0) ^b	0.10 (<0.1) ^a	12.5 (2.5) ^b	39 (3) ^a
Reference (mean)	5.4 (2.7)	0.18 (0.1)	26.8 (12.1)	50 (14.4)

Copper-lithium and zinc-lithium ratio in sediment

The ratios of zinc to lithium in the sediment were significantly higher at reference sites than at farm sites (ANOVA; $F_{1,20}=7.307$, $p=0.014$), however, these differences were driven by the high ratio at Holmes Inlet (Figure 2.4). No significant differences were detected in the ratios of copper to lithium between farm and reference sites (ANOVA; $F_{1,20}=0.176$, $p=0.68$), and the ratios at Holmes Inlet, Millar Channel and Atleo overlapped (Figure 2.4). Differences were not attributable to the proximity of sites to salmon farms.

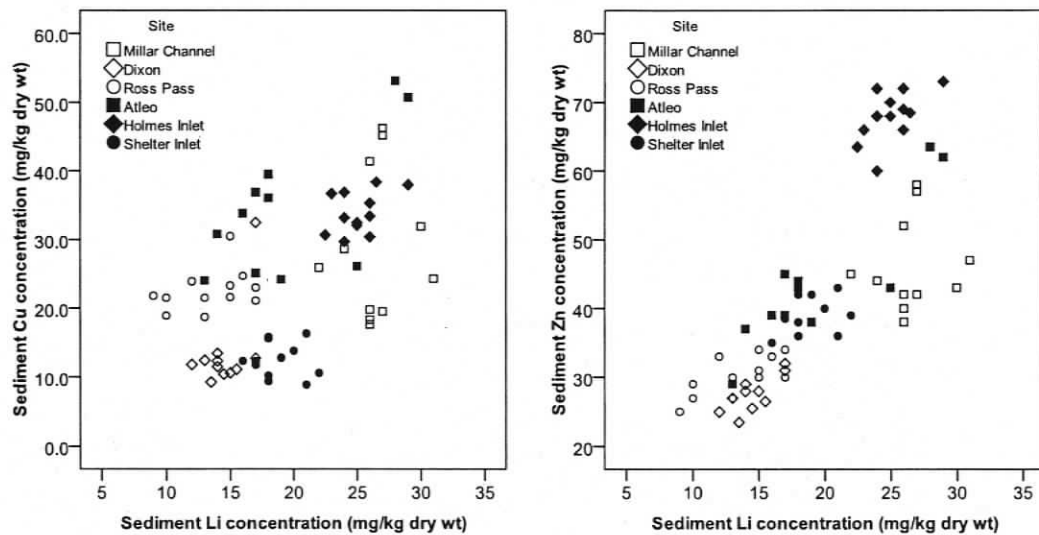


Figure 2.4. Sediment copper and zinc to lithium ratios at farm and reference sites. Open symbols are farm sites, filled symbols are reference sites.

Discussion

Sources of variation in the metal concentration and tissue weight of clams

The concentrations of metals detected in salmon feed were similar to those detected by other researchers and suggest that salmon farms continue to be a source of metals to adjacent sediment and biota. Based on the concentrations of metals detected in the feed and the volume of feed and feces released into the environment annually, I calculated that 3.4 tonnes of zinc and 675kg of copper were released province-wide from salmon feed and feces in 2006; zinc was also released via leaching from cage structures (Burrige 2003) and copper from antifouling paints (Cross 2005; Dean et al. 2007). In 2007, there were 132 licensed salmon farms, thus the quantity of metals release from individual farms was relatively small. In comparison, sewage outfalls from Macaulay and Clover Point in Victoria released 4.5 tonnes of copper and 2.9 tonnes of zinc in 2006 (CRD 2007). Although the quantity of metals released from individual salmon farms is small when compared to sources such as sewage waste, researchers have previously detected significantly elevated concentrations in biota and sediment within several hundred metres of salmon farms (Chou et al. 2002; Chou et al. 2003; Dean et al. 2007). Metal levels tend to drop considerably within a few hundred metres of farms (Cross 2005), yet even contaminants at low levels have the potential to biomagnify and pose health risks. Manila clams are good candidates for monitoring metal contaminants in coastal waters and are known to biomagnify contaminants (Ji et al. 2006).

When I examined metal concentrations in Manila clams and sediment situated from 500 to 700m from farms I did not detect farm-derived effects. Increased concentrations of mercury have been detected in rockfish situated at similar distances from salmon farm sites to those in my study (deBruyn et al. 2006) and have been attributed in part to enriched species abundance and biomass near salmon farms, which can increase the trophic level and hence contaminant concentrations in nearby predator species. While enhanced trophic position may increase metal concentrations in rockfish, Manila clams are primary consumers and the results of this study suggest they were not affected in a similar way.

Differences were detected in the metal concentrations in Manila clams between farm and reference sites, but they were primarily attributable to the values detected in clams from Holmes Inlet and Shelter Inlet. Clams from these two sites showed similar patterns of accumulation of arsenic, cadmium, and copper, while clams from the other four sites had very similar levels of arsenic and cadmium. This suggests that the distinction between farm versus reference did not explain as much of the variability between sites as other factors. Both the Holmes Inlet and the Shelter Inlet sites were at the end of protected channels and differed from the other sites in this respect (Figure 2.1). There are currently 22 active salmon farms within Clayoquot Sound (BC Ministry of Agriculture and Lands 2007) which limited the number of reference sites available for this study. Holmes Inlet and Shelter Inlet were chosen despite differences from the other sites in terms of exposure to wind, waves, and deposition, because they met two important criteria for reference sites in this study, which were that salmon farms had not been sited there previously and that the beaches were actively harvested by First Nations. Ideally, both clams and sediment would be analysed for metals before farms were operational to eliminate the difficulty in finding properly matched reference sites in areas with high densities of salmon farms and to facilitate monitoring changes in concentration over time.

In agreement with other studies (Boyden 1974; Bebianno et al. 1993; Riget et al. 1996; Usero et al. 1997; Baudrimont et al. 2005; Ji et al. 2006), the tissue arsenic and cadmium concentrations increased with shell length, indicating that Manila clams are unable to regulate these metals (Table 2.3). At Holmes Inlet and Shelter Inlet, the rate of arsenic accumulation also accelerated in the larger clams, possibly due to a reduction in growth rates. At these two sites, the large clams generally had lower tissue weights than clams of the same length from the other sites (Figure 2.3). Slower growth rates have been associated with increased metal concentrations in tissue (Luoma and Rainbow 2005) and slow growing bivalves in pristine areas can have metal concentrations comparable to rapidly growing conspecifics in polluted areas (Leung and Furness 1999; Leung and Furness 2001).

This study found that the copper and zinc concentrations in tissue decreased with increasing body size (Table 2.3), although this effect was not consistent throughout the seasons or at all the sites (Appendix B). Other researchers have also found decreasing copper and zinc concentration with shell length (Boyden 1974; Leung and Furness 1999), which may be explained by higher metabolic function and growth rates in small individuals and a concurrent dilution of metals (Leung and Furness 1999).

Monthly variations were also detected in tissue arsenic and cadmium concentrations; these metals consistently increased with shell length, although the slope of the regressions varied between months (Appendix B). Month to month variation can sometimes result from seasonal changes in precipitation which influences ambient metal concentrations. Periods of high precipitation and run-off can increase the load of suspended materials in coastal waters and elevate both soluble and particulate metal concentrations (Fowler and Oregioni 1976). Monthly changes in precipitation at each site were not monitored in this study. The west coast of Vancouver Island typically experiences the highest precipitation from October through January (Environment Canada 2008). The metal concentrations in the tissue of Manila clams were generally not higher in these months (Appendix B); however, it could take some time for the concentrations in tissue to track the changes in the external environment. Alternately, precipitation may not have significantly affected metal concentrations. Manila clams may not have consumed food particles associated with terrestrial run-off because they are highly selective feeders and preferentially retain particulate organic matter of marine rather than terrestrial origin (Kasai et al. 2004).

Upwelling is also a factor in seasonal changes in metal concentrations in bivalves. It brings metals that are normally distributed at intermediate depths, such as cadmium, to the surface (Lares and Orians 1997; Lares et al. 2002). This results in short term variability in metal concentrations in tissue that are closely associated with the timing of upwelling, which occurs primarily in late spring through summer off the coast of

Vancouver Island (Ianson et al. 2003; Sackmann et al. 2004). Clams were not collected in the summer in this study because First Nations do not typically harvest at this time. At Millar Channel and Dixon, an increased cadmium concentration was observed in clams of all sizes collected in April; however, this effect was not apparent at the other four sites.

Farm-derived waste can provide nutrient subsidies to clams (Shpigel and Fridman 1990; Mazzola and Sara 2001) and clams from farm sites did have greater tissue weight than clams of the same shell length from reference sites (Table 2.5 and Figure 2.3). Although these differences were fairly modest and inconsistent between sites and months, they suggest that clams at the farm sites may be consuming farm waste. Differences among sites in temperature may also have been partially responsible for the observed differences in tissue weight. Temperature can affect the duration of spawning, the metabolic rates of clams, and food availability (Holland and Chew 1974; Beninger and Lucas 1984; Dowd 1997; Ojea et al. 2004). Fluctuations in tissue mass can affect the concentration of metal burdens in Manila clams (Ojea et al. 2004; Ji et al. 2006), thus, the asynchronous fluctuations within the small geographic area in this study suggest that metal concentrations in clams were not directly comparable between sites.

No relationship was apparent between a higher tissue weights and lower metal concentrations (i.e., biodilution; Appendix B and C). Cossa et al. (1980) did not detect evidence of biodilution and suggested that monthly fluctuations in metal concentration within a site were not straightforward and resulted from several biochemical changes induced by reproduction and seasonal adaptations (e.g., changing lipid levels (Beninger and Lucas 1984)).

Despite year-round dispersal of farm waste into the marine environment and the observation of slightly greater tissue weight in clams collected from sites near farms, no differences were detected in the variability of metal concentrations between clams from farm and reference sites. Alternative sources of metals may have had a greater influence on concentrations in tissue and obscured farm- derived influences. Since no differences

were detected in the variability in clam tissue weights between farm and reference sites, fish farms may have provided only marginal dietary subsidies rather than the bulk of the Manila clam diet.

With the exception of cadmium, metal concentrations in Manila clams from Clayoquot Sound were within the same range as those detected by the Canadian Food Inspection Agency (CFIA), as well as those found in Korea, China and Spain (Table 2.5). Cadmium was detected at levels almost twice as high as though reported by the CFIA; however, these levels were limited to four samples consisting of the largest clams collected in April and October from Holmes Inlet and one sample of large clams from Shelter Inlet. Clams from Holmes Inlet were also enriched in $\delta^{15}\text{N}$ compared to large clams from other sites, which may have been attributable to periodic starvation (Chapter 3). If food availability was sometimes reduced, bivalves would have slow growth rates and this can result in elevated metal concentrations (Leung and Furness 1999; Leung and Furness 2001).

Health Canada does not have any guidelines for metal concentrations in bivalves. Both the FDA and the US EPA have guidelines. The US EPA guidelines are more conservative and are intended to protect ethnic and subsistence fishers who consume more fish products than the general population and who also repeatedly harvest from the same local water bodies (US EPA 2000). These can be used as a rough guide when determining whether the concentrations found in this study warrant further investigation.

The highest arsenic concentrations were reached in large clams from Holmes Inlet and Shelter Inlet at 6.7 and 5.8mg/kg wet weight, respectively (Appendix B), while the mean concentration in legal sized clams (i.e., those consumed by Ahousaht residents; 38mm shell length) from all sites was 3.2mg/kg wet weight. The toxicity of arsenic is largely dependent on the chemical species. In BC, the predominant species in Manila clams is the organic form, arsenobetaine, which is much less toxic than inorganic arsenic (Cullen and Dodd 1989). Values for the percent inorganic arsenic in Manila clams in BC and

neighbouring Puget Sound areas are reported to be less than 1% (Cullen and Dodd 1989; Johnson and Roose 2002; US EPA 2003), thus the maximum and the average calculated value of inorganic arsenic in Manila clams would be 0.067 and 0.032mg/kg wet weight, respectively. According to the US EPA guidelines (2000), clam consumers in Ahousaht would be restricted to 0.5 to 1 clam meal per month. However, in this study, as in Johnson and Roose's study (2002), the levels detected in reference areas bracket those of the areas under investigation, and probably all reflect background concentrations. Furthermore, consuming clams is an integral part of the Ahousaht lifestyle, and provides multiple cultural, health, and economic benefits. The US EPA cautions that seafood consumption is more than a dietary choice for aboriginal people and it can not simply be eliminated if the concentrations of metals do not fall within current guidelines.

Concerns have arisen over the cadmium levels in BC oysters (3-4 mg/kg wet weight) that exceeded the export guidelines for shellfish, which range from 1 to 2 mg/kg wet weight, depending on the country (Kruzynski 2004). Clams from Holmes Inlet and Shelter Inlet approached these guidelines with maximum concentrations in the large bivalves reaching 0.82 and 0.85mg/kg, respectively (Appendix B). These levels trigger consumption guidelines of 3 clam meals per month according to the US EPA non-cancer health endpoints (2000). The cadmium bioavailability (determined in part by the metal species), and the smoking habits and nutritional status of the consumer must all be considered before any health risks can be assessed.

Copper and zinc concentrations fell within the range of those detected in Manila clams in other studies (Table 2.5). The lower concentration of these essential metals in larger clams suggested that these metals were regulated and were not at elevated levels (Boyden 1974; Colombo et al. 1997).

Sources of variation in the metal concentration in sediment

Despite very similar patterns of arsenic, cadmium, and zinc accumulation in the clams from Holmes Inlet and Shelter Inlet, the concentrations of these three metals were elevated in the sediment at Shelter Inlet but not at Holmes Inlet (Figure 2.2; Table 2.7).

Sediment is not a major food source of Manila clams (Kasai et al. 2004), although it can be important due to resuspension (Chong and Wang 2000; Fan and Wang 2001). Neither this study nor Ji et al. (2006) found a close correlation between sediment and metal concentrations in Manila clams. However, only the total metal concentration in the sediment was evaluated in my study and by Ji et al. (2006), and this may have provided a poor indication of the metal bioavailability and uptake, which is significantly impacted by the metal species, the sediment concentration of iron oxides and organics, the pH, and the reduction/oxidation potentials (Bryan and Langston 1992; Fan and Wang 2001).

Variation among sites in the concentration factor from sediment to clams could result from differences in the proportion of metals that were assimilated from the dissolved phase as opposed to ingested metals associated with food particles. Metal bioavailability from ingested food is termed the assimilation efficiency and is influenced by the relative quantities of sediment, phytoplankton and bacteria in the food, as well as the presence of acid volatile sulfide in sediment (Chong and Wang 2001), and can vary by an order of magnitude under natural conditions (Lee and Luoma 1998). When the assimilation efficiency from food is low, dissolved cadmium and zinc have been shown to contribute as much as 40 and 60%, respectively, to the total metal accumulation in Manila clams (Chong and Wang 2001) and dissolved cadmium can be a major source of cadmium for bivalves in British Columbia (Lekhi et al. 2008).

Salinity significantly affects the uptake rates from the dissolved phase; a 50% reduction in salinity can increase zinc and cadmium uptake rates in Manila clams up to three-fold (Chong and Wang 2001). Reduced salinity associated with increased run-off in winter and spring precipitation events may increase the uptake of zinc and cadmium significantly. The seasonal changes in the concentrations in clam tissue observed in this study may have been due in part to seasonal fluctuations in salinity, and not solely to changes directly in the metal concentration in the sediment.

At Holmes Inlet, the interim marine sediment quality guideline (ISQG) for arsenic (7.24mg/kg dry weight (Canadian Council of Ministers of the Environment 2002)) was exceeded, and at Millar Channel, Ross Pass, Atleos Beach and Holmes Inlet, the copper guideline (18.7mg/kg dry weight (Canadian Council of Ministers of the Environment 2002)) was exceeded (Table 2.7). However, these values may reflect natural sediment concentrations as the cadmium, copper and zinc concentrations were all well below the baseline values of sediment samples collected from unpolluted, coastal BC waters (Harding and Goyette 1989). Natural sediment metal concentrations can vary by up to an order of magnitude and are affected by surface chemistry, reduction/oxidation reactions, and desorption processes that are associated with the release of metals from sediments that are undersaturated with sulfides (Luoma 1990).

The metal concentrations in sediment showed the greatest variability at Millar Channel and Atleos Beach, indicating that the seasonal shifts were due largely to site-specific processes (Appendix D). Millar Channel and Atleos Beach were the most exposed to open ocean currents (Figure 2.1); they were well flushed and may have been more susceptible to changing environmental conditions. In winter months, for example, higher rainfall, rougher seas, higher tides and increased wind effects can all increase turbulence (Buggy and Tobin 2006), resuspending sedimentary particulate matter and increasing its availability for ingestion by clams.

Copper-lithium and zinc-lithium ratio in sediment

The elevated zinc to lithium ratios in the sediment collected from reference sites were attributable to the high zinc to lithium ratios at Holmes Inlet, suggesting that some anthropogenic input of zinc may have occurred into this area (Figure 2.4). The zinc concentrations in the sediment were also the highest at Holmes Inlet (68mg/kg dry weight; Table 2.6) but were still well below the ISQG of 124mg/kg dry weight (Canadian Council of Ministers of the Environment 2002). The copper to lithium ratios were similar at farm and reference sites and no significant differences were detected. Copper and zinc released from farms may elevate concentrations in sediment primarily within ~300m of salmon farms. Sutherland et al. (2007) only found elevated levels of zinc at three of 70

far-field stations (300-500m and 1-5km) and did not find any evidence of increased copper at far-field stations. Dean et al (2007) found that copper and zinc concentrations reached background levels within 300m of salmon farms. Farm sites in this study were situated between 500 and 700m from salmon farms.

Conclusions

Although metal concentrations in salmon feed suggested that salmon farms could be a source of arsenic, cadmium, copper and zinc, this study did not detect evidence that farms were elevating metal levels in either clam tissue or sediment at intertidal beaches near farms. However, clams from farm sites had marginally greater tissue weights than clams from reference sites which suggested that farm waste may have provided a source of nutrients. No differences were detected in the variability of tissue weights and metal concentrations between clams from farm and reference sites and the concentrations in both the clam tissue and the sediment did not suggest a significant anthropogenic input in the area. This was further supported by the absence of anomalous copper and zinc to lithium ratios at farm sites.

In accordance with the findings of previous researchers, the arsenic and cadmium concentrations increased with shell length while the copper and zinc concentrations decreased, although this effect was not consistent throughout the seasons. Metal concentrations in Manila clams from Clayoquot Sound were generally within the same range as those detected by the Canadian Food Inspection Agency (CFIA) and probably reflected background concentrations. The findings suggested that differences in metal concentrations in Manila clams were primarily due to site specific attributes rather than the presence or absence of farm waste. Ideally future studies would determine baseline metal concentrations in clams and sediment before salmon farms are established and monitor changes over time to eliminate the difficulty in finding properly matched farm and reference sites.

Chapter 3 . Nitrogen and carbon stable isotopes in Manila clams and sediment near salmon farms in Clayoquot Sound, BC

Abstract

Salmon farms in British Columbia (BC) discharge organic waste directly into the coastal environment and whether this waste negatively impacts local marine life is currently not well known. First Nations in isolated coastal communities rely heavily on marine foods and have expressed concerns that bivalves are being negatively impacted by the influx of waste. Protein and oil derived from small pelagic fish are key ingredients in salmon feed, and waste feed and feces from farms is typically enriched in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ compared to marine particulate organic matter (POM). Bivalves that incorporate farm waste into their diet may consequently have enriched tissue relative to bivalves that primarily consume marine POM. To test these hypotheses, I analysed salmon feed, sediment and bivalves from three intertidal shellfish beds between 500 and 700m from fish farms and from three reference sites 1 to 10km from active farms in Clayoquot Sound, BC, for stable isotopes of nitrogen and carbon. Contrary to findings in earlier studies, salmon feed was not a useful tracer of farm waste because it was not enriched in ^{15}N and ^{13}C relative to marine POM. Salmon feed was depleted in ^{15}N relative to feed collected in BC only a few years earlier. Clams from reference sites were enriched in ^{15}N and ^{13}C compared to clams from farm sites; however, site had greater explanatory power than proximity to salmon farms. No effects of salmon farms were detected on the sediment $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, or the variability of the isotopic signature of clams and sediment. I suspect that the reformulation of salmon feed with greater proportions of terrestrial rather than marine sources of protein and oil reduced the utility of isotopic signatures for tracing farm waste. The proportion of marine-derived protein and oil in salmon feed will likely continue to decrease due to rising prices and static catch of pelagic fish.

Introduction

Salmon farming is a rapidly growing global industry, producing in excess of 1.4 million tonnes of fish world-wide in 2006, and more than doubling its output in a ten year period (Tacon and Metian 2008). Canada is the fourth largest producer of farmed salmon, following only Norway, Chile, and the United Kingdom. The industry was established in

British Columbia (BC) in the 1970s and the majority of Canadian salmon is now reared on the west coast. In 2006, BC produced over 70 000 tonnes of salmon (Department of Fisheries and Oceans Canada 2009). Using a feed conversion ratio (the mass of feed required for an equivalent increase in body mass) of 1.2 (Cross 2005), this required over 82 000 tonnes of feed. According to Cross' estimates, approximately 15% of salmon feed applied to netpens is subsequently released into the water column; 8% in dissolved form and 7% as solid wastes (feed and feces), which may either settle beneath netpens or be transported away from farms as suspended particulates in the water column.

First Nations from the Broughton Archipelago have reported that beaches situated near salmon farms are showing evidence of eutrophication and that the clams from these beaches are smaller and darker in colour (Routledge et al. 2007). Brooks et al. (2004) have documented reduced mollusc populations and sediment quality within 100m of the Carrie Bay salmon farm in the Broughton Archipelago. It is difficult to determine the exact cause of the changes to clams; however, a useful first step may be to determine if clams near farms are consuming farm waste.

The effects of salmon farm-associated organic waste on the coastal marine environment have primarily been studied by evaluating the characteristics of sediment in the areas around netpens. To date, research has focussed on the effects of farms on sediment characteristics such as the carbon and nitrogen content, metal contamination, and sediment particle size (Holmer 1991; Chou et al. 2002; Kempf 2002; Chou 2004; Pereira et al. 2004; Mendiguchia 2006). However, local hydrodynamics greatly influence the extent to which farm waste will disperse into the surrounding marine habitat (Gowen and Bradbury 1987; Holmer 1991; Brooks et al. 2004; Sara et al. 2006). The aforementioned techniques are relatively insensitive for detecting impacts beyond 50 to 100m of farms.

Farm-derived nutrients have been detected at distances of up to 1000m away using biomonitors, such as the seagrass *Posidonia oceanica*, which suffers reduced growth rates and eventual shoot mortality (Marba et al. 2006). With increasing frequency, stable

isotopes are also being used to trace the dispersal of farm-derived nutrients (Grey et al. 2004; deBruyn et al. 2006; Sara et al. 2006; Kutti et al. 2007). At sites with high current velocities, they can detect organic loading in sediment at distances of 300 to 1000m from fish farms (Sara et al. 2004; Vizzini et al. 2005; Sara et al. 2006; Yokoyama et al. 2006; Holmer et al. 2007). This is due to the influx of protein and oil derived from marine fish which enrich the sediment beneath and adjacent to farms in ^{15}N and ^{13}C (McGhie et al. 2000; Gao et al. 2006; Kutti et al. 2007). Hence, stable isotopes provide an additional metric to evaluate the effects of fish farms on the surrounding benthic environment that might be more sensitive than bulk sediment analyses and, contrary to biological indicators, can be used in widely different geographic zones.

Stable isotopes can be useful tracers of farm waste because organic matter derived from farms tends to be isotopically distinct from other marine sources. Fishmeal and oil derived from small pelagic fish have been the key ingredients in salmon feed, and the tissues of bivalves that consume farm waste have been enriched in stable isotopes. $\delta^{15}\text{N}$ of mussels from a fish farm in Hong Kong was $10.5 \pm 0.3\text{‰}$ and $\delta^{13}\text{C}$ was $-19.3\text{‰} \pm 0.3\text{‰}$, an enrichment of almost 2‰ and 1.3‰, respectively, when compared to mussels from reference sites (Gao et al. 2006). These differences were attributed to the consumption of fish feces and pelleted fish feed.

The objectives of this study were to determine whether stable isotopic analysis could be used to trace farm waste in sediment and bivalves on intertidal beaches near salmon farms. Manila clams were chosen as the study organism because they are important in the coastal native diet (Marlor and Eyding 2005), are known to consume farm waste (Shpigel and Fridman 1990), and reach isotopic equilibrium with their diet within about two months (Yokoyama et al. 2005). $\delta^{15}\text{N}$ shows a stepwise enrichment averaging $3.4 \pm 1.1\%$ with each successive step in the food chain (Minagawa and Wada 1984), which results from the retention of the isotopically heavier ^{15}N and the excretion of the lighter ^{14}N during amino acid synthesis (Adams and Sterner 2000). The heavier isotope of carbon

fractionates to a lesser degree than nitrogen, approximately 1.0 to 1.5‰, thus the ratio in an organism reflects its food source plus a slight enrichment (DeNiro and Epstein 1978).

Manila clams primarily consume marine POM and benthic microalgae, as well as small quantities of terrestrial organic matter (Kasai et al. 2004), although they will consume farm-derived organic waste when it is available (Shpigel and Fridman 1990; Mazzola and Sara 2001). A study conducted in Clayoquot Sound in 2001 to 2003 (Hahn 2004) determined that the mean $\delta^{15}\text{N}$ of salmon feed at that time was approximately 12‰ and another study from the same time period determined $\delta^{15}\text{N}$ in salmon feed of six to 12mm diameter was 9.9 to 11.0‰ (Petersen et al. 2005). Petersen et al. (2005) found that $\delta^{13}\text{C}$ ranged from -22.0 to -21.3‰. On the west coast of Vancouver Island, $\delta^{15}\text{N}$ of marine POM ranges from 7.7 to 10.2‰, while $\delta^{13}\text{C}$ ranges from -25.3 to -22.0‰ (Wu 1997). I expect Manila clams on beaches near farms to be enriched in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ compared to clams from reference sites.

The salmon production cycle at farms encompasses 18 to 24 months (Cross 2005), thus, farms potentially release organic matter with a constant isotopic signature year round. In comparison, the isotopic signature of clams and sediment from reference sites is influenced by autochthonous POM, which changes seasonally (Kasai et al. 2004). If the isotopic signature of farm waste is considerably different to autochthonous sources of POM, the signature of sediment from farm sites could be more stable than at reference sites. I do not expect this year round source of waste to have a stabilizing effect on stable isotopes of clam tissue, which are fairly consistent year round despite variations in the signature of their food sources (Kasai et al. 2004).

Farm waste can be incorporated into the coastal ecosystem in several different ways, either via primary producers, detritivores, suspension feeders, or carnivores. This study will contribute to our understanding of the dispersal of farm waste further afield and increase our knowledge of its path into the marine environment via suspension feeders.

Methods

Field methodology is described in Chapter 2. In addition to sediment and clam samples, three feed samples from Clayoquot Sound were analysed; one from Millar Channel, one from Dixon Bay and one from near Herbert Inlet. Pellet size ranged from 6 to 12mm. These farms, as well as the farm at Ross Pass, are operated by Mainstream, and the assumption was made that all of the farms under the same management use the same feed. Feed was stored in coolers in new borosilicate glass jars while in the field, then frozen at -20°C until analysed for stable isotopes of nitrogen (N) and carbon (C).

A significant positive correlation has been detected between weight and $\delta^{15}\text{N}$ in fish *Microstomus pacificus* (Spies et al. 1989), and between age and $\delta^{15}\text{N}$ in clams *Mya truncata* (Atwell et al. 1998), and a significant negative correlation between size and $\delta^{13}\text{C}$ in clams *Macoma balthica* (Rossi et al. 2004). Therefore, a range of sizes of clams were used to evaluate the effect of fish farms. Clam shell length was measured using Vernier callipers and from each site clams of 29, 39, and $48 \pm 1.5\text{mm}$ length were analysed in triplicate for stable isotopes of N and C. Clams were rinsed in distilled water, a small piece of the foot tissue ($\sim 0.1\text{g}$) was excised for isotopic analysis and the remaining clam tissue was homogenized and analysed for metal concentrations (Chapter 2).

Lipids in tissue can skew the results of stable isotope analysis because lipid synthesis discriminates against the heavier isotope of carbon and results in depleted tissue ^{13}C compared to lipid-poor tissue (DeNiro and Epstein 1978). The foot tissue was analysed because lipids form only a minor component of the foot (4.4 to 5.0%) and this percentage is constant throughout the year (Ojea et al. 2004).

Tissue and sediment samples were stored at -20°C until analysis, and then freeze dried at -45°C in a Freezone 12L Freeze Dry System (Labconco, Kansas City, MI). Sediment samples were gently crushed and sieved to obtain a fine fraction (0 to $200\mu\text{m}$). It would have been preferable to examine a smaller sediment size fraction, such as $<20\mu\text{m}$, to bracket the size range of food particles Manila clams ingest (Fan and Wang 2001), however, the quantity of sediment was insufficient.

Clam and sediment samples were ground to a fine powder using an agate mortar and pestle and packed in tin capsules (Metal Microanalysis Ltd, Okehampton, UK). For each new sample, all surfaces and tools were cleaned with 95% ethanol. Samples were combusted to generate CO₂ and N₂ gas. Isotope ratio in the evolved gas was measured with a Thermo Finnigan Delta Advantage Isotope Ratio Mass Spectrometer fitted with a Costech Metal Combustion System (Valencia, CA). The precision of the mass spectrometer for $\delta^{15}\text{N}$ is $\pm 0.3\text{‰}$ and for $\delta^{13}\text{C}$ is $\pm 0.2\text{‰}$. With every twelve samples run, one replicate and one standard consisting of either fish muscle tissue for tissue samples ($\delta^{13}\text{C} = -21.9\text{‰}$, $\delta^{15}\text{N} = 10.7\text{‰}$) or Buffalo River Sediment for sediment samples ($\delta^{13}\text{C} = -19.7\text{‰}$, $\delta^{15}\text{N} = 3.8\text{‰}$) were used as standards. The machine was calibrated with every batch using two standards in which the ^{15}N and ^{13}C bracketed that of the samples (Caffeine $^{15}\text{N} = -12.4\text{‰}$ and $^{13}\text{C} = -29.8\text{‰}$, and Dogfish liver $^{15}\text{N} = 15.6\text{‰}$ and $^{13}\text{C} = -17.8\text{‰}$). Samples were analysed for stable nitrogen isotope ratio ($\delta^{15}\text{N}$) and stable carbon isotope ratio ($\delta^{13}\text{C}$).

Data analyses

Analysis of covariance (ANCOVA) was used to test for differences in tissue $\delta^{15}\text{N}$ between farm and reference sites, over multiple months, and in clams of a range of shell lengths. Analysis of variance (ANOVA) was used to test for differences in tissue $\delta^{13}\text{C}$, and in sediment $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ between sites over multiple months. Residuals were visually inspected to confirm that normality and homogeneity of variance assumptions were not violated. Differences between months were compared individually at each site using ANOVA and the Bonferroni method was used to correct for multiple comparisons. All statistical procedures were performed using SPSS 15.0 software and in all cases, $p < 0.05$ was considered significant.

To determine if the variability in clam $\delta^{15}\text{N}$ differed between farm and reference sites over the sampling period (April 2005 until February 2006), regressions of $\delta^{15}\text{N}$ against the shell length were plotted and the residual variance at farm and reference sites was

compared using ANOVA. Differences in the amount of variation in clam $\delta^{13}\text{C}$, and in sediment $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ between farm and reference sites were tested by comparing the coefficients of variation using ANOVA.

Results

Sources of variation in the isotopic signature of clam tissue

The mean nitrogen signature of salmon feed collected at the study sites was 9.4‰ (SD=0.67, n=3), which was depleted relative to values of 9.9 to 11.0‰ (six to 12mm feed (Petersen et al. 2005)) and 10.8 to 13.1‰ (Hahn 2004) detected in BC salmon feed several years earlier. No significant differences in $\delta^{15}\text{N}$ in salmon feces relative to feed have been detected (McGhie et al. 2000), so I estimated that the nitrogen isotopic signature of the bulk of the waste coming from farms was similar to the feed signature, approximately 9.4‰.

$\delta^{15}\text{N}$ in clam tissue was significantly lower at farm sites than at reference sites (ANCOVA; $p < 0.001$, $F_{1,188} = 53.61$; Table 3.1). The mean differences in $\delta^{15}\text{N}$ between farm and reference sites were very small (~0.3‰) and were primarily attributable to the values at Holmes Inlet, where mean $\delta^{15}\text{N}$ was greater than at the other five sites in all size clams (Figure 3.1). Mean $\delta^{15}\text{N}$ in large clams (48mm) from Holmes Inlet, for example, was 10.6‰, while the mean $\delta^{15}\text{N}$ in large clams at the other five sites ranged from 9.9 to 10.2‰.

$\delta^{15}\text{N}$ increased significantly with shell length and was significantly different between the sampling months (ANOVA; $p < 0.001$ for both; Table 3.1), but the differences between months were only significant at Dixon (ANCOVA, Bonferroni adjusted p -value for multiple comparisons was 0.003, $p = 0.001$, $F_{3,29} = 6.89$; Appendix E). At Dixon, $\delta^{15}\text{N}$ was depleted throughout the size range in both October and December relative to the other months. At the other sites, $\delta^{15}\text{N}$ varied between months, however, the differences observed were not significant.

The mean carbon signature of salmon feed was -21.1‰ ($SD=0.30$, $n=3$), which was similar to values of -22.0 to -21.3‰ detected by Petersen et al. (2005). Salmon feces is enriched in ^{13}C relative to salmon feed (McGhie et al. 2000; Kutti et al. 2007) by an average of 0.8‰ (Ye et al. 1991; McGhie et al. 2000), so I estimated that $\delta^{13}\text{C}$ value of the feces released in the study area would be approximately -20.3‰ .

$\delta^{13}\text{C}$ in clam tissue was significantly lower at farm sites than at reference sites (ANCOVA; $p=0.002$, $F_{1, 188}=126.89$), and was primarily driven by enriched clams collected from Holmes Inlet and Shelter Inlet (Figure 3.2). At Ross Pass, larger clams were significantly more enriched in $\delta^{13}\text{C}$ than smaller clams (ANOVA; $F_{2,3}=12.436$, Bonferroni adjusted p -value for multiple comparisons was 0.003 , $p=0.001$), while at the other sites the differences between small and large clams varied widely and were not significant (Appendix E). Significant differences in $\delta^{13}\text{C}$ between months were detected at Millar Channel, Ross Pass and Atleos Beach (ANOVA using Bonferroni adjusted p -value= 0.003 ; $p < 0.001$, $F_{3, 29}=8.18$; $p < 0.001$, $F_{3, 29}=9.63$ and $p=0.001$, $F_{3, 29}=7.8$, respectively). At Millar Channel and Ross Pass, April differed significantly from October, and at Ross Pass and Atleos Beach, April differed significantly from December.

Examination of the residual plots suggested that an important variable affecting $\delta^{13}\text{C}$ was unaccounted for by the model at all sites. No significant differences were detected in the variability of clam tissue $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ between farm and reference sites (ANOVA; $p=0.18$, $F_{1, 4}=2.63$ and $p=0.23$, $F_{1, 4}=1.96$, respectively).

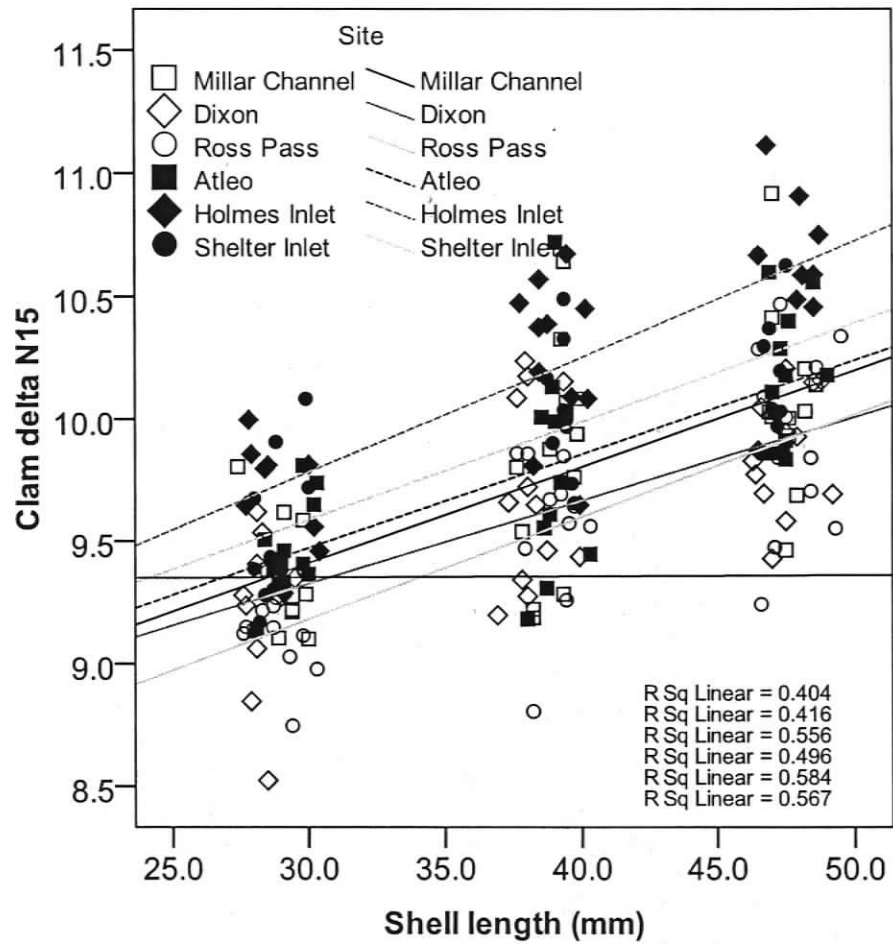


Figure 3.1. $\delta^{15}\text{N}$ in Manila clams collected from three sites near salmon farms and three reference sites in Clayoquot Sound, BC. Open symbols are farm sites and filled symbols are reference sites. The horizontal line depicts $\delta^{15}\text{N}$ detected in salmon feed collected from the study area; $\delta^{15}\text{N}$ of salmon feces was presumed to be similar.

Table 3.1. Evaluation of the differences in $\delta^{15}\text{N}$ in Manila clam tissue (n=194; the shell lengths of clams evaluated were 29, 39, and 48mm \pm 1.5) collected from three farm and three reference sites in April, October, and December, 2005, and February, 2006, using ANCOVA. Residuals were visually inspected to confirm that normality and homogeneity of variance assumptions were not violated.

	Df	F	Sig.	Observed Power
Farm treatment	1	53.61	<0.001	1.00
Month	3	9.56	<0.001	1.00
Shell length (mm)	1	182.05	<0.001	1.00
Error	188			

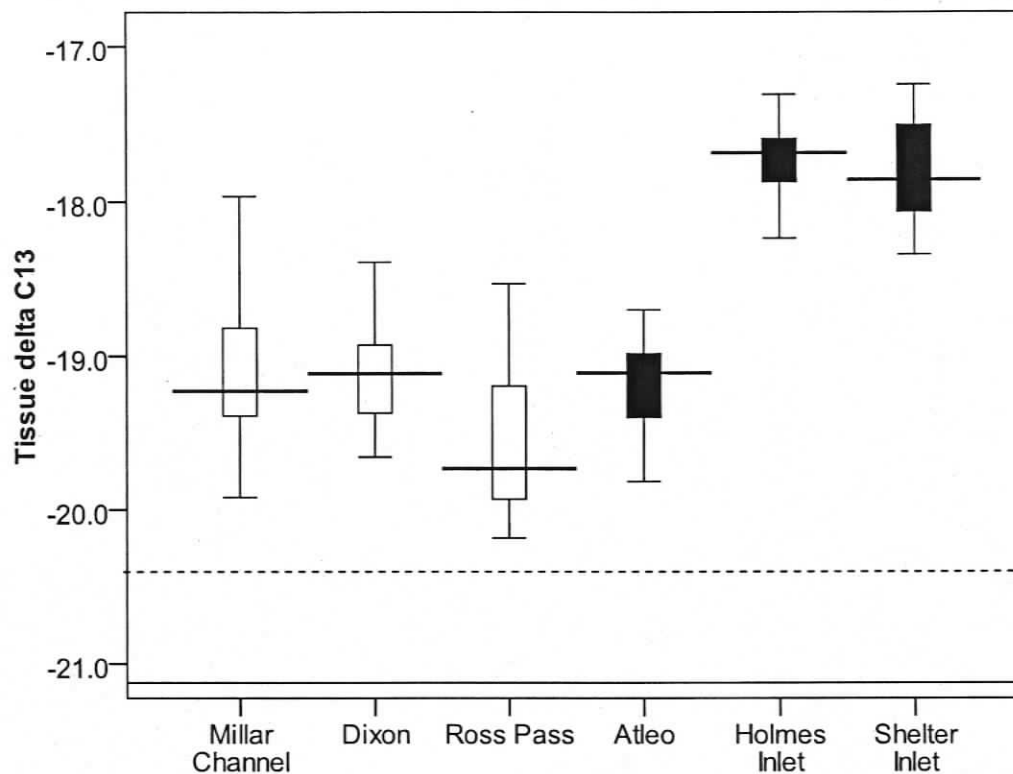


Figure 3.2. $\delta^{13}\text{C}$ in Manila clams ($n=194$) collected from three farm and three reference sites in Clayoquot Sound, BC. Open boxes are farm sites and filled boxes are reference sites. The solid horizontal line depicts $\delta^{13}\text{C}$ detected in salmon feed collected from the study area and the dashed horizontal line depicts the estimated value of $\delta^{13}\text{C}$ of salmon feces (salmon feces was assumed to be enriched by $\sim 0.8\text{‰}$ relative to salmon feed).

Sources of variation in the isotopic signature of sediment

Mean $\delta^{15}\text{N}$ in the sediment was significantly higher at farm sites than at reference sites ($\delta^{15}\text{N}=7.6\text{‰}$, $\text{SD}=0.9$ and $\delta^{15}\text{N}=7.0\text{‰}$, $\text{SD}=0.9$, respectively; $p=0.013$, $F_{1,3}=6.591$; Table 3.2); however, site had greater explanatory power than distinctions between farm and reference. Sediment $\delta^{15}\text{N}$ was depleted at Millar Channel and Holmes Inlet relative to the other sites in every month sampled (Figure 3.3). Differences between Dixon, Ross Pass, Atleos Beach, and Shelter Inlet depended on the month. In both April and December, mean sediment $\delta^{15}\text{N}$ was similar at Dixon and Ross Pass and slightly more depleted at

Atleos Beach and Shelter Inlet. In October and February, sediment $\delta^{15}\text{N}$ was similar within and differed between these four sites. Mean sediment-tissue fractionation differed between sites and ranged from 1.7‰ at Ross Pass to 4.5‰ at Holmes Inlet; mean-sediment tissue fractionation at all sites was 2.9‰ (SD = 1.0).

Mean sediment $\delta^{13}\text{C}$ was the same at farm and reference sites ($\delta^{13}\text{C} = -22.2\text{‰}$, SD=2.1 and $\delta^{13}\text{C} = -22.2\text{‰}$, SD=1.3). Significant differences occurred between months in $\delta^{13}\text{C}$ in sediment (Figure 3.4 and Table 3.4). Sediment from Ross Pass and Shelter Inlet was more enriched in $\delta^{13}\text{C}$ than the other four sites in every month. There was little variation within and between months in the sediment $\delta^{13}\text{C}$ at Millar Channel, Dixon and Holmes Inlet, and at Atleos Beach it was within the same range as these three sites but the fluctuations within and between months was greater. Sediment-tissue fractionation differed between sites and ranged from 0.2‰ at Ross Pass to 7.5‰ at Shelter Inlet; mean sediment-tissue fractionation for all sites was 4.1‰ (SD=2.4).

No differences were detected between farm and reference sites in the variability of sediment $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (ANOVA of coefficients of variation $p=0.95$, $F_{1,4}=0.004$ and $p=0.77$, $F_{1,4}=0.100$, respectively).

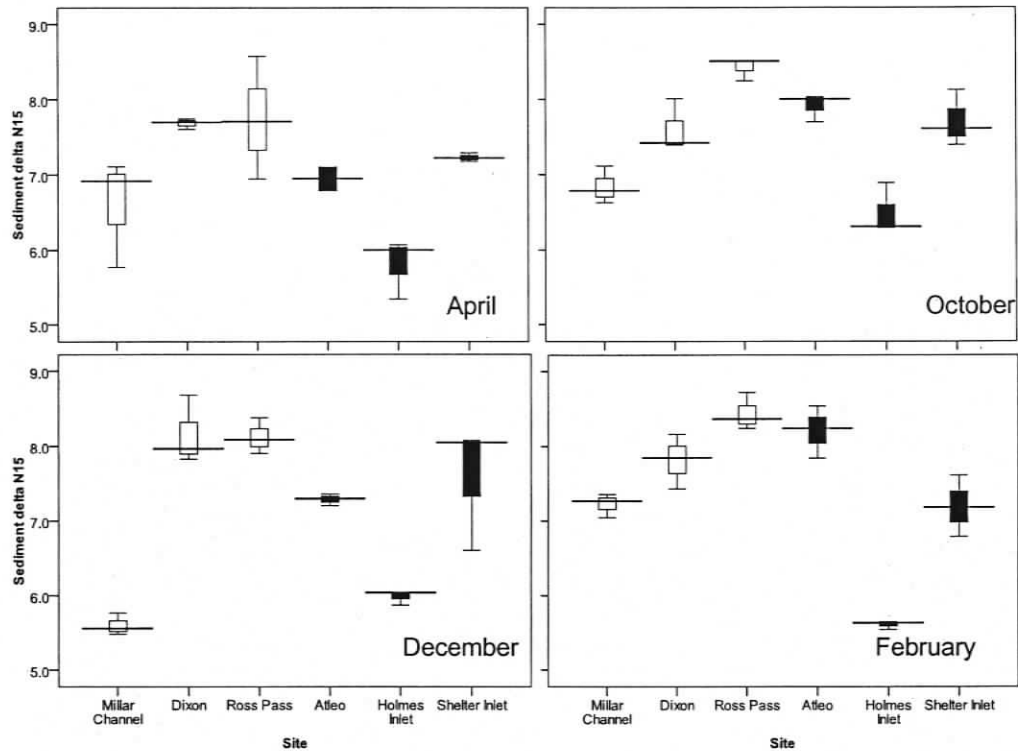


Figure 3.3. $\delta^{15}\text{N}$ in sediment collected in four sampling months from three farm and three reference sites in Clayoquot Sound, BC. Open boxes are farm sites and filled boxes are reference sites.

Table 3.2. Evaluation of the differences in $\delta^{15}\text{N}$ in sediment ($n=3$ at each site and in each sample month) collected from three farm and three reference sites in April, October, and December, 2005, and February, 2006, using ANOVA. Residuals were visually inspected to confirm that normality and homogeneity of variance assumptions were not violated.

Source	df	F	Sig.	Observed Power
Farm treatment	1	6.59	0.013	0.72
Month	3	1.18	0.326	0.30
Error	64			

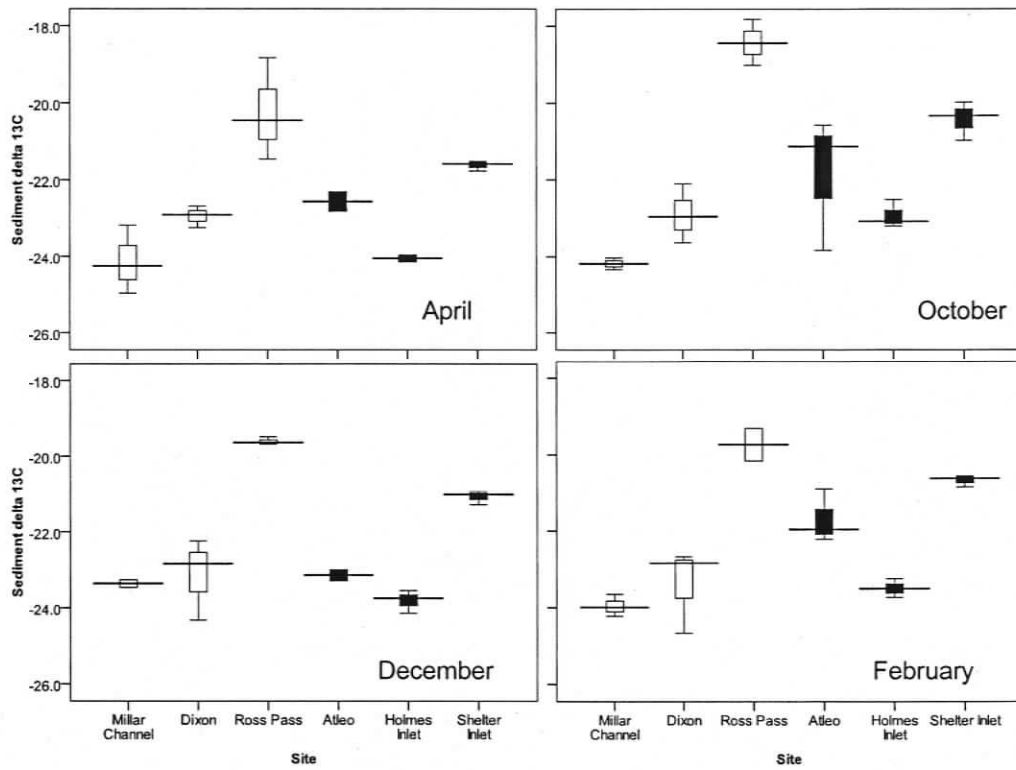


Figure 3.4. $\delta^{13}\text{C}$ in sediment collected in four sampling months from three farm and three reference sites in Clayoquot Sound, BC. Open boxes are farm sites and filled boxes are reference sites.

Table 3.3. Evaluation of the differences in $\delta^{13}\text{C}$ in sediment ($n=3$ at each site and month) collected from three farm and three reference sites in April, October, and December, 2005, and February, 2006, using ANOVA. Residuals were visually inspected to confirm that normality and homogeneity of variance assumptions were not violated.

Source	Df	F	Sig.	Observed Power
Farm treatment	1	0.00	1.000	0.05
Month	3	0.51	0.676	0.15
Error	64			

Discussion

Sources of variation in the isotopic signature of clam tissue

I expected that the isotopic signature of farm waste would be significantly enriched relative to the primary food sources of Manila clams, and that the consumption of farm waste would enrich the nitrogen signature of clam tissue at farm sites. The nitrogen isotopic signature of the salmon feed collected in 2005 in this study was 9.38‰ (SD=0.3, n=3), and was depleted relative to salmon feed collected a few years earlier by Hahn (2004) and Petersen et al. (2005).

Salmon are carnivorous and feed for farmed salmon has traditionally been formulated to meet their dietary requirements using fishmeal and oil of small pelagic fish. In 1995, the aquaculture industry used approximately 28% and 34% of global fishmeal and oil supplies, respectively (Tacon and Metian 2008). By 2005, the proportion of fishmeal and oil used by the aquaculture industry had increased to 69% and 94%, respectively, and salmon feed is one of the top consumers of these limited resources (Tacon and Metian 2008). Faced with a finite supply and rising prices, manufacturers of salmon feed are substituting marine protein and oil with sources of terrestrial origin. Globally, the average proportion of fishmeal in feed was estimated at 45% in 1995 and dropped to 35% by 2005, while the proportion of fish oil also declined from about 25% in 1995 to 21% in 2005, and this trend is expected to continue (Tacon and Metian 2008). The proportion of fishmeal and oil used in salmon feed also varies by country. Some countries, such as England, have stricter rules pertaining to the use of animal by-products in feed (Tacon 2005).

Terrestrial protein and oil have depleted isotopic signatures compared to marine sources (Craig 1953; Chisholm et al. 1982; Yokoyama et al. 2006). In the year I collected salmon feed (2005), Skretting manufactured Canadian feed using poultry meal, feather and fat, as well as ingredients derived from corn and wheat (Appendix A). The ingredients are listed in order of quantity on feed bags, although the exact proportion of each ingredient is

proprietary information. The ingredients in the feed were unavailable from the study by Hahn (2004) and Petersen et al. (2005), but differences in the quantity of marine versus terrestrial sources of protein and oil may have been responsible for the decrease I observed in $\delta^{15}\text{N}$ of salmon feed.

I estimated that the nitrogen isotopic signature of salmon feces was similar to the feed signature (McGhie et al. 2000), thus the signature of the bulk of the waste coming from farms was approximately 9.4‰. Kasai et al. (2004) calculated that the diet of Manila clams is 55% marine POM, 33% benthic microalgae, and 12% terrestrial POM. I did not measure $\delta^{15}\text{N}$ of marine POM on the west coast of Vancouver Island; however, Wu (1997) determined it falls within 7.7 to 10.2‰; $\delta^{15}\text{N}$ of benthic microalgae ranges from nine to 10‰ (Sakamaki and Richardson 2009); and of terrestrial POM is approximately 0.6‰ (Kasai et al. 2004). Due to the reformulation of fish feed and the overlap of its isotopic signature with marine POM, which is the primary food source of Manila clams, and benthic microalgae, stable isotopic analysis was not useful for following the path of farm waste in this study.

It is good practice to use another tracer in addition to stable isotope analysis whenever more than two food sources are present (Fry and Sherr 1984). In addition to metal analysis, the original study design included analysis of persistent organic pollutants (POPs), which can complement stable isotope analysis when assessing feeding ecology (Fisk et al. 2002). This part of the study was not completed but it may have posed problems similar to those encountered with isotopic analysis because the majority of POPs released from fish farms originate in the marine fish oil component of feed (Hites et al. 2004). It would be interesting to determine whether POP concentrations in feed have also declined.

Fatty acid analysis has been used in conjunction with stable isotopic analysis to trace the dispersal of farm waste in sediment and bivalves (McGhie et al. 2000; Gao et al. 2006) and is another tool that may be useful for tracing farm waste despite the

reformulation of feed. Fatty acid patterns in marine primary producers may be passed conservatively to primary consumers (Dalsgaard et al. 2003). The fatty acids profiles of marine protein and oil sources are distinctly different from those of terrestrial origin (Seierstad et al. 2009); therefore, the patterns in clams that consume farm waste may be distinct from those in clams that consume only autochthonous POM. Another option would be to trace farm waste indirectly by evaluating changes in community composition and population numbers. The availability of waste feed and feces near netpens can increase fish species richness and abundance (Dempster et al. 2002; Valle et al. 2007), and this effect has not yet been investigated in BC.

Clams from reference sites were very slightly enriched in $\delta^{15}\text{N}$ compared to clams from farm sites and these differences were mainly due to values in clams from Holmes Inlet. Clams in Holmes Inlet may have undergone periodic starvation. When food is unavailable, nitrogen uptake is near zero while lean tissue is catabolized and nitrogen is excreted at a reduced rate (Adams and Sterner 2000), incidentally enriching tissue in $\delta^{15}\text{N}$ (Hobson et al. 1993; Adams and Sterner 2000; Yokoyama et al. 2005). Clams rely primarily on marine POM, largely composed of phytoplankton, and benthic microalgae; both are limited by light, nutrients, and temperature (Sverdrup 1953; Li et al. 2000). Yokoyama et al. (2005) found that the $\delta^{15}\text{N}$ in Manila clam tissue was enriched by approximately 0.6‰ after one month of starvation, while $\delta^{13}\text{C}$ was not significantly effected. Clams from Holmes Inlet had lower tissue weights, although these differences were modest and inconsistent between months and shell lengths (Chapter 2).

$\delta^{15}\text{N}$ increased significantly with shell length at all sites in all months, which was consistent with the significant positive relationship detected between clam age and $\delta^{15}\text{N}$ by Atwell et al. (1998). They hypothesized that either larger clams consume food that belongs to a higher trophic level, or that a physiological change in the clam itself is responsible. Rossi et al. (2004) also found a significant correlation between tissue isotopic values and shell length in *Macoma balthica* and attributed this to size-dependent differences in feeding modes. Smaller clams rely entirely on suspension feeding because

they must bury at shallower depths in order for their siphon to reach the surface, while adults are able to bury deeper in the sediment and use a combination of both deposit and suspension feeding.

Few temporal changes in $\delta^{15}\text{N}$ were identified in clams, and were only significant at Dixon, where clams were depleted in October and December relative to April and February. These findings are consistent with those of Kasai et al. (2004), who did not detect significant temporal changes in $\delta^{15}\text{N}$ in Manila clams despite significant fluctuations in the isotopic signature of marine POM. Manila clams have relatively slow tissue turnover times and are sedentary making them useful integrators of highly variable isotopic values of primary producers and consumers (Fukumori et al. 2008).

$\delta^{13}\text{C}$ of the salmon feed collected from the study sites was -21.1‰ and was very similar to the signature of feed from the Broughton Archipelago, BC, collected in 1999 (-21.4 to -22‰) (Sutherland et al. 2001) and detected by Petersen et al. (2005) of -22.0 to -21.3‰ ; enriched compared to feed from Norway (-24‰) (Kutti et al. 2007); and depleted compared to feed from Australia (-18.2 to -18.5‰) (McGhie et al. 2000). The estimated $\delta^{13}\text{C}$ value of the feces released in the study area was -20.3‰ , which was consistent with the value determined by Ye et al. (1991) of -20.48‰ . The carbon isotopic signature of marine POM on the west coast of Vancouver Island averages -25.3 to -22.0‰ (Wu 1997), for benthic microalgae between -19 to -16‰ (Sakamaki and Richardson 2009), and for terrestrial POM between -30 to -23‰ (Sutherland et al. 2001). $\delta^{13}\text{C}$ of the feed and feces released from the farm was not greatly enriched relative to marine POM and depleted relative to benthic microalgae and was not a useful tracer of farm waste. Clams collected from reference sites were significantly enriched in $\delta^{13}\text{C}$ compared to clams from farm sites but the differences were driven by values at Holmes Inlet and Shelter Inlet. The food available to clams in these two inlets may have been enriched in $\delta^{13}\text{C}$ relative to the other sites.

Differences between small and large clams in $\delta^{13}\text{C}$ generally varied widely among sites and seasons and were not significant; however, at Dixon in February and at Holmes Inlet in December, larger clams were significantly more enriched than smaller clams. Size-specific feeding modes can lead to intra-specific differences in $\delta^{13}\text{C}$ (Rossi et al. 2004); however, the relationship between clam size and $\delta^{13}\text{C}$ differed between months and sites suggesting that size was not the only determinant of $\delta^{13}\text{C}$. Examination of the residual plots suggested that an important explanatory variable was unaccounted for by the model at all sites.

$\delta^{13}\text{C}$ in Manila clam tissue is fairly stable over time, although heavy precipitation can deluge the marine environment with terrestrial POM and result in temporarily depleted carbon isotopic signatures (Kasai et al. 2004). Significant differences in $\delta^{13}\text{C}$ between months were detected at Millar Channel, Ross Pass and Atleos Beach. At Millar Channel and at Ross Pass, clams were significantly depleted in October compared to April, and at Ross Pass and at Atleos Beach, clams were significantly depleted in December compared to April. Over the sampling period, weather stations at Tofino, BC (approximately 20km southeast of the study sites at Ahousaht), reported the heaviest rains in October 2005 and in January 2006 (513 and 617mm, respectively).

No significant differences were detected in the variability of clam tissue $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ between farm and reference sites. Unless large quantities of food with dramatically different isotopic signatures were available, no change would be expected. Kasai et al. (2004) found that the isotopic signature of Manila clam tissue remained fairly stable despite significant changes in the composition and isotopic signature of ambient POM.

Differences in stable nitrogen and carbon isotopes between clams from farm and reference sites were primarily due to enriched values in clams from Holmes Inlet and Shelter Inlet. In a study in Clayoquot Sound that evaluated differences in biomass and stable nitrogen isotopes of mussels between farm and reference sites, Hahn (2004) found that interlocation was the greatest source of variation. The results of this study are

consistent with those findings and suggest that when possible, reference sites should be located within the same inlet or channel as farm sites. In the year this study was conducted (2005-2006), two farms had recently been active just north of Flores Island, and one farm south east of Dixon Bay (Figure 2.1). Few sites were available in the study area that were located in the same channel as salmon farms and that had not recently been exposed to farm waste.

Sources of variation in the isotopic signature of sediment

The mean sediment $\delta^{15}\text{N}$ was significantly higher at farm sites than at reference sites; however, site had greater explanatory power than distinctions between farm and reference (Table 3.2 and Figure 3.3). The sediment at Millar Channel and Holmes Inlet was depleted in $\delta^{15}\text{N}$ relative to the other sites in every month sampled. Holmes Inlet is at the end of a long narrow channel while Millar Channel is more exposed site to oceanic currents; both beaches receive freshwater inputs as do Shelter Inlet, Atleos Beach, and Dixon (Figure 2.1). Neither clams nor sediment were collected within 3m of fresh water streams; however, periodic deposition of terrestrial matter may have occurred after precipitation events via fresh water streams or run-off.

In both April and December, mean sediment $\delta^{15}\text{N}$ was similar at Dixon and Ross Pass and was slightly depleted at Atleos Beach and Shelter Inlet in comparison (Figure 3.3). Perhaps the material deposited on these four beaches during these months had comparable isotopic signatures. In October and February, sediment $\delta^{15}\text{N}$ was similar within and differed between Dixon, Ross Pass, Atleos Beach, and Shelter Inlet, suggesting that the material deposited on the beaches in October was similar to that deposited in February.

The mean clam-sediment fractionation ranged from 1.7 to 4.6‰ over the sampling months and sites and deviated at all sites, except Millar Channel, from the established fractionation value in Manila clams of 3.4 to 3.6‰ (Yokoyama et al. 2005). This is not unexpected as Manila clams consume resuspended sediment (Chong and Wang 2000) but it is not a dominant food source (Kasai et al. 2004). The sediment samples contained

particles up to 200 μ m and Manila clams typically ingest particles smaller than \sim 7.5 μ m so many of the particles present in the samples would have been rejected as pseudofeces (Defosse and Hawkins 1997). Manila clams are primarily suspension feeders (Kasai et al. 2004) and some components of their diet may not have settled into the sediment.

In an Australian study, salmon feed and feces were enriched in ^{13}C by \sim 7‰ relative to sediment from reference sites and the sediment near salmon farms was significantly enriched (McGhie et al. 2000). In the present study, the isotopic signature of the feed and the estimated value of salmon feces released from farms were only slightly enriched relative to published values for marine POM, and were depleted relative to benthic microalgae. Thus, farm waste was not expected to have a detectable influence on the isotopic signature of nearby intertidal sediment in this study. Current estimates of fishmeal and oil use indicate that Australian salmon feed is manufactured using even less fishmeal and oil than Canadian feed (Tacon and Metian 2008). The reformulation of salmon feeds worldwide is likely reducing the utility of stable isotopes for detecting the presence of farm waste.

Sediment from Ross Pass and Shelter Inlet was more enriched in $\delta^{13}\text{C}$ than the other four sites in every month, which may have been the result of different proportions of marine POM, benthic microalgae and terrestrial POM at each site. As with nitrogen isotopic analysis, the mean sediment-tissue fractionation for carbon was 4.1‰ and deviated from the established diet-tissue fractionation for Manila clams of +0.6 to +0.9‰ (Yokoyama et al. 2005). The sediment samples contained particles larger than those typically consumed by Manila clams and some food sources may not have settled on to the sediment. Differences in fractionation among sites may have also been due to differences between sites in the availability of benthic microalgae and suspended POM in the marine habitat, which can influence the source of dietary carbon in Manila clams (Kanaya et al. 2005).

Because farm waste was not enriched relative to the isotopic signature of ambient POM, it was not expected to have a stabilizing influence on sediment isotopic signatures at farm sites and none was detected.

Conclusion

The stable nitrogen and carbon isotopic signatures of feed were not significantly enriched compared to autochthonous sources of organic matter and thus were not useful tracers of farm waste in the study area. This was likely the result of the reformulation of salmon feed with protein and oil of terrestrial rather than marine origin. Differences in stable isotopic signatures were driven by enriched $\delta^{15}\text{N}$ in clams from Holmes Inlet and enriched $\delta^{13}\text{C}$ in clams from Holmes Inlet and Shelter Inlet; values at the other four sites were similar. Mean $\delta^{15}\text{N}$ in sediment at farm sites was slightly enriched compared to reference sites, although site again had greater explanatory power than the distinction between farm and reference. No differences were detected in mean sediment $\delta^{13}\text{C}$ or in variability of tissue and sediment isotopic signatures between farm and reference sites.

According to Cross' estimate (2005), I calculated that over 12 000 tonnes of waste feed and feces were released from fish farms in BC in 2006. This waste could be traced using fatty acid patterns, which are conserved from primary producer to primary consumer (i.e., clams). Fatty acid profiles of clams consuming farm waste may differ from those in reference areas despite feed reformulation. Future studies could also trace waste indirectly by evaluating changes in community composition and population numbers. The availability of waste feed and feces near netpens can increase fish species richness and abundance (Dempster et al. 2002; Valle et al. 2007), and this effect has not yet been investigated in BC.

Chapter 4 . General discussion

Salmon farming is highly controversial in British Columbia (BC), and the many documented and perceived threats can lead to great concern in the communities where farms are situated (Hamouda et al. 2005). It is also an important commercial industry, and in 2006 BC produced over 70 000 tonnes of farmed salmon, almost triple the quantity of commercially caught landings of wild salmon (Department of Fisheries and Oceans Canada 2009). The Ahousaht First Nations live in Clayoquot Sound on the west coast of Vancouver Island, BC, and in 2006 there were 22 active salmon farms in the area (BC Ministry of Agriculture and Lands 2007). Ahousaht is a relatively isolated community plagued by high unemployment rates (Stackhouse 2001), and clams are a nutritious, economical food, as well as an important part of the traditional lifestyle and diet.

First Nations have expressed concerns that salmon farms have negatively impacted nearby clams and beaches and that, despite their observations that bays nearby are negatively affected, both government researchers and scientists study only the impacts immediately beneath farms (Routledge et al. 2007). Metal concentrations in sea urchins and lobsters in eastern Canada were elevated close to fish farms (Chou et al. 2002; Chou et al. 2003); however, the concentrations in nearby intertidal clams had not yet been investigated.

The objective of this study was to determine whether waste released from salmon farms was elevating the concentrations of metals in Manila clams and sediment on nearby beaches. This study did not find evidence that waste released from the farms elevated the metal concentrations in bivalves and sediment above background levels. The levels detected in clams and sediment from fish farms bracketed those from reference areas and probably reflected background concentrations. The mean concentrations of arsenic and cadmium in clams from all sites were slightly elevated and may have represented natural and historic levels, as was suspected with cadmium in BC oysters (Kruzynski 2004).

Clam and sediment samples were also analysed for stable isotopes of nitrogen and carbon to trace farm waste. Clams utilize multiple sources of organic matter, including marine particulate organic matter (POM), benthic microalgae, and terrestrial POM. These all have depleted nitrogen and carbon signatures compared to pelagic marine fish (Yokoyama et al. 2006; Noriyuki et al. 2007), which were the primary ingredients in salmon feed until recently (Tacon and Metian 2008). In 1996, marine derived protein and oil constituted almost 70% of feed ingredients, yet by 2006, these ingredients comprised only 50% of salmon feed (Tacon and Metian 2008). Earlier studies detected enriched carbon and nitrogen isotopic values in sediment and organisms near salmon farms due to an influx of marine fishmeal and oil (McGhie et al. 2000; deBruyn et al. 2006; Gao et al. 2006) and I initially expected that the waste feed and feces released from farms would be significantly enriched in ^{15}N and ^{13}C . I found that the nitrogen and carbon isotopic signatures of salmon feed were not distinctly different from the usual food sources of Manila clams and this was likely due to the reformulation of salmon feeds (Tacon 2005; Yokoyama et al. 2006). The reformulation of salmon feed with terrestrial protein and oil sources is a world wide phenomenon (Tacon and Metian 2008) and has probably reduced the utility of stable isotopes as a tracer of salmon farm waste.

Organochlorine contaminant (OC) concentrations could also have been used to complement stable isotope analysis and assess feeding ecology (Fisk et al. 2002), and were initially part of the study design. Studies have detected elevated concentrations of OC, which are lipid soluble and concentrated in fish oil, in salmon feed (Easton et al. 2002; Hites et al. 2004). Since feed has been reformulated, the OC concentrations in feed have likely been reduced as well (Carlson and Hites 2005). Poultry meal and feather meal contain more copper and zinc than fishmeal, thus the concentration of metals in feed may actually increase as feeds continue to be reformulated (Chou et al. 2002).

Fatty acid profiles of fish feed and feces have been used in conjunction with stable isotopic analysis to trace the dispersal of farm waste in sediment (McGhie et al. 2000). Fatty acid patterns can be passed conservatively from primary producers to primary

consumers (Dalsgaard et al. 2003), and the patterns in clams that eat farm waste may be significantly different to patterns in clams that consume autochthonous POM. Fatty acid analysis may continue to be a useful tracer despite feed reformulation and could be evaluated in future studies.

Much of the variation in metal concentrations and stable isotopes in clams and sediment in this study were attributable to site-specific differences. Due to the high density of salmon farms in Clayoquot Sound, finding reference sites that had never been exposed to farm waste and had similar features to those of farm sites was difficult. In this study, two of the three reference sites were situated at the end of long, narrow channels and differed from the other four sites in this respect, which may have increased the intra-site variability and obscured differences in sediment and tissue metal concentrations and stable isotope values. Clams from the inlets had higher arsenic and cadmium concentrations and lower zinc concentrations than clams from the other four sites, and were enriched in ^{15}N and ^{13}C compared to clams from the other sites. This was consistent with Hahn's (2004) findings in Clayoquot Sound that the differences in stable isotope signatures of intertidal eelgrass, *Fucus* and mussels were greater between inlets and channels than between farm and reference sites.

Sediment and biota samples would ideally be collected in an area before a salmon farm was established, particularly if a farm was situated near a beach that was actively harvested by First Nations. If contaminant levels could be compared before and after a farm was in operation, the difficulty in finding properly matched reference sites would be minimized and changes in concentrations over time could also be monitored.

Researchers were able to work closely with First Nations from Ahousaht which facilitated the selection of the study organism and study sites. No successful partnerships were developed between researchers in this project and salmon aquaculturalists, although researchers attempted to collaborate with industry partners and meet requirements that were conditional to working together. I suspect that the highly controversial nature of

salmon farming in BC (Hamouda et al. 2005) hindered the ability of stakeholders and researchers to work co-operatively.

Health Canada does not have any guidelines for metal concentrations in bivalves. Metal toxicity is largely related to speciation and considering the importance of seafood to the physical and cultural health of coastal native people, guidelines should be developed that pertain to the metal species that pose a health risk. Coastal First Nations consume more seafood than the national average and harvest repeatedly from the same waterbodies; therefore, these guidelines should be especially protective of First Nations and other sensitive subpopulations (US EPA 2000).

It was important to investigate the potential of farm-related contaminants to elevate metal concentrations in Manila clams. Fears among First Nations about contamination can lead to a decrease in consumption of traditionally harvested foods and these foods are often replaced with nutrient poor substitutes (Chan and Receveur 2000). Manila clams are low in fat and high in protein, and are an excellent source of essential fatty acids. They contain 0.67g/ 100g, as opposed to protein sources such as chicken, beef, and pork, which have no detectable fatty acids (King et al. 1990).

Researchers have previously identified farm-derived metals in the marine environment (Chou et al. 2002; Chou et al. 2003; Chou 2004; deBruyn et al. 2006) and because these metals could accumulate over time (deBruyn et al. 2006), ongoing monitoring of clams from Clayoquot Sound would be warranted. Future studies should also investigate the concentrations of persistent organic pollutants in feed, and if levels have not declined despite the reformulation of feed (Easton et al. 2002; Jacobs et al. 2002; Hites et al. 2004), clams and intertidal sediment should also be examined. Organic contaminants in feed are transferred to the sediment beneath net pens in the form of waste feed and feces (Hellou et al. 2005) and whether they are subsequently transferred to local biota is currently unknown.

Salmon farming has numerous other environmental impacts which were not investigated in this study. These include the effect of escaped salmon on wild populations (McGinnity et al. 1997; Gross 1998; Youngson 2001; Weir and Grant 2005), the impact of the pesticides and antibiotics used on farms to control diseases and parasites (Jacobsen and Berglind 1988; Ervik et al. 1994; Weston 1996; Burridge et al. 2004), the reduction of world fish supplies that results from using pelagic fish to feed farmed fish (Naylor et al. 1998; Pauly et al. 1998; Naylor et al. 2000; Weir and Grant 2005), and the transmission of sea lice from farmed salmon to wild salmon (Morton et al. 2004; Westcott et al. 2004; Krkosek et al. 2005; Krkosek et al. 2007). Although the proportion of fishmeal and oil in salmon feed has decreased, the equivalent of almost five tonnes of pelagic fish are still required to produce one tonne of farmed salmon (Tacon and Metian 2008).

In 2007, salmon returns at the study site in Dixon Bay were negligible; only 17 salmon returned. Plankton tows collected from Dixon Bay in January 2008 indicated that the waters around the salmon farm were heavily infested with sea lice (Morton 2008). As a precautionary measure, the Ahousaht First Nations postponed the renewal of the Dixon farm license in 2008 (Dart 2008; Drews 2008). Although sea lice are naturally found on adult salmon in the open ocean, heavy infestations on farmed salmon can pose serious risks to juvenile salmon when they migrate from freshwater to nearshore marine waters (Peet 2007). The Ahousaht were given further cause for concern in 2008 when 2 500 Atlantic salmon escaped from one of the farms in Clayoquot Sound (Drews 2008).

The Ministry of Agriculture and Lands is currently responsible for licensing salmon farms and protects them from liability under the Right to Farm Act. Recently the Supreme Court ruled that salmon farms are actually a fishery rather than a form of agriculture and as such fall under the federal authority of the Department of Fisheries and Oceans (DFO) and not the Ministry of Agriculture and Lands (Hawkes 2008; Brown 2009). Unless this decision is appealed, fish farms will fall under federal authority within twelve months. This may change the way that salmon farms in BC are managed because

DFO is also responsible for wild salmon stocks, and these appear to be negatively impacted by sea lice originating on farmed fish (Morton et al. 2004; Krkosek et al. 2005; Krkosek et al. 2007).

The rearing of aquatic organisms, including seaweed, shellfish, crustaceans and finfish, has been practiced by humans for millennia. In BC, the Kwak'waka'wakw people of the Broughton Archipelago constructed intertidal clam gardens to facilitate the easy harvest of clams and to enhance productivity (Harper et al. 1995; Glavin 2003) and in China, agriculture and livestock production have been combined with fish cultivation for 2500 years (Tacon 1996). Farmers grow grass at pond borders that feed both livestock and carp, and stock filter feeders and molluscivores to utilize the pond's other resources (Chen et al. 1995). Farming carnivorous fish is comparatively new and energy-intensive, requiring heavy inputs of time, money, pelagic fish resources, and technology. The numerous environmental impacts of rearing carnivorous fish such as salmon are not fully understood and require further investigation.

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Appendix A. Ingredients in salmon feed manufactured by Skretting in 2005.

Six and 9mm Skretting Vitalis SA salmonid feed pellets were analysed. The ingredients for 9mm pellets are given below. Fishmeal doubled in price from 2005 to 2006, and prices increases are leading to increases in the use of terrestrial sources of protein and oil (Tacon and Metian 2008).

The proportion of marine versus terrestrially-derived protein and oil varies with the size of the pellets; marine ingredients are found in greater quantities in starter and finishing feeds (Tacon and Metian 2008). The ingredients for smaller pellets (1.5mm) are presented for comparison. The ingredients are listed in order of quantity on feed bags, although the quantity of each ingredient is proprietary information. The salmon feed for smaller fish (i.e., 1.5mm pellets) contains more poultry meal and replaces some of the fish oil component of feed with poultry fat.

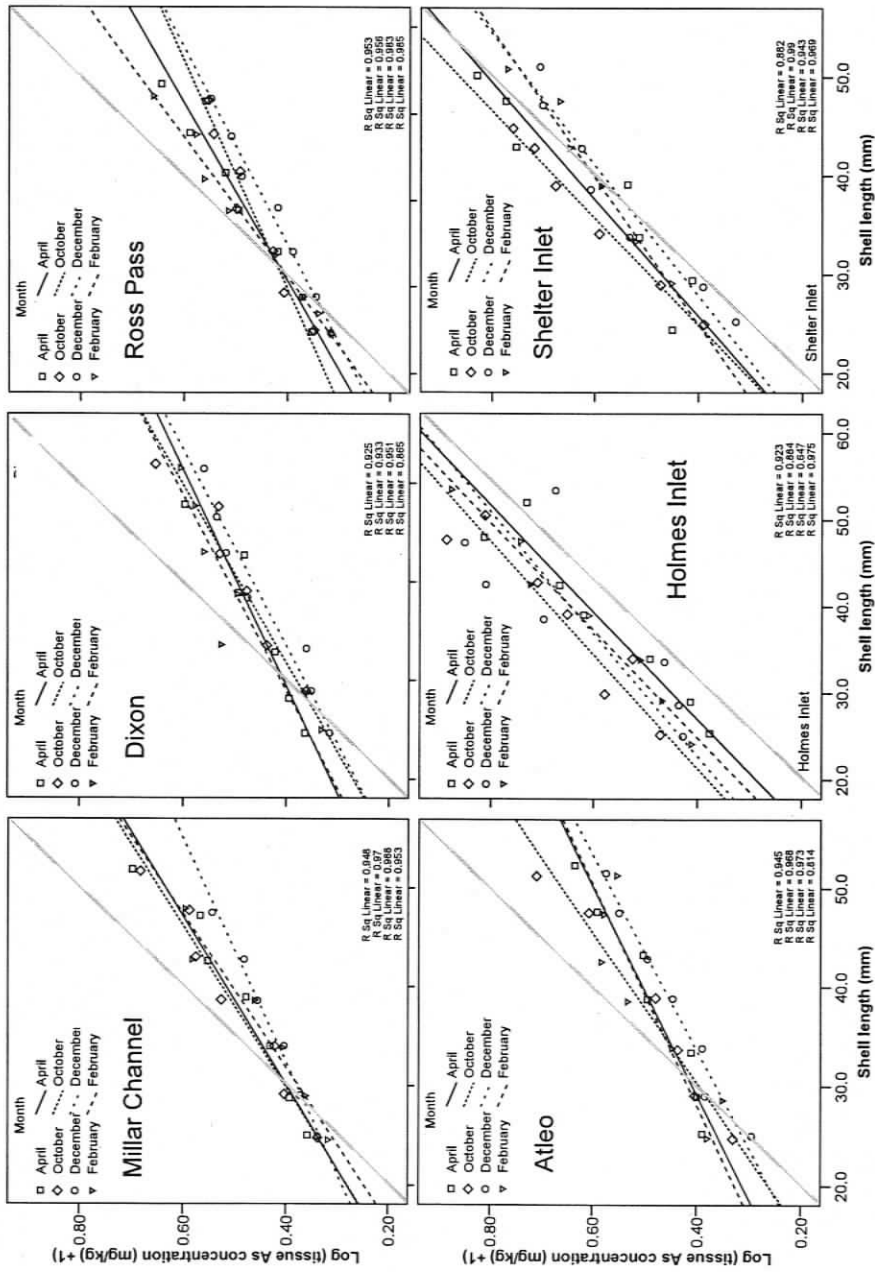
Ingredients (9.0 mm pellets)

Fish meal, fish oil, poultry meal, corn gluten meal, whole wheat, betaine, squid meal, brewers yeast, soy lecithin, Vit E, ethoxyquin, Vitamin and mineral premix

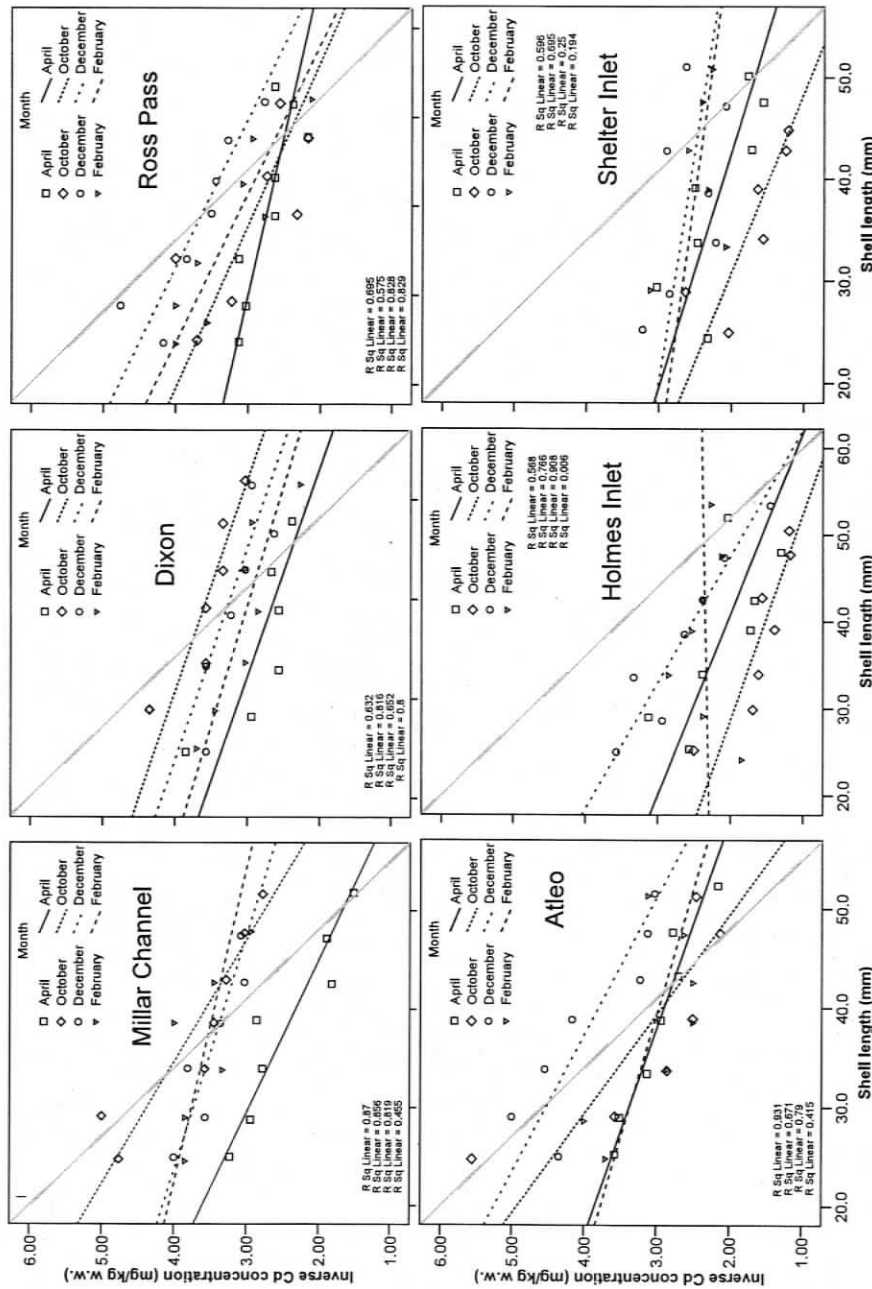
Ingredients (1.5 mm pellets)

Fish meal, poultry meal, fish oil, corn gluten meal, whole wheat, poultry fat, feather meal, poultry fat (*written as on ingredients list, with poultry fat included twice*), brewers yeast, Vit E ethoxyquin (antioxidant) a vitamin premix, mineral premix

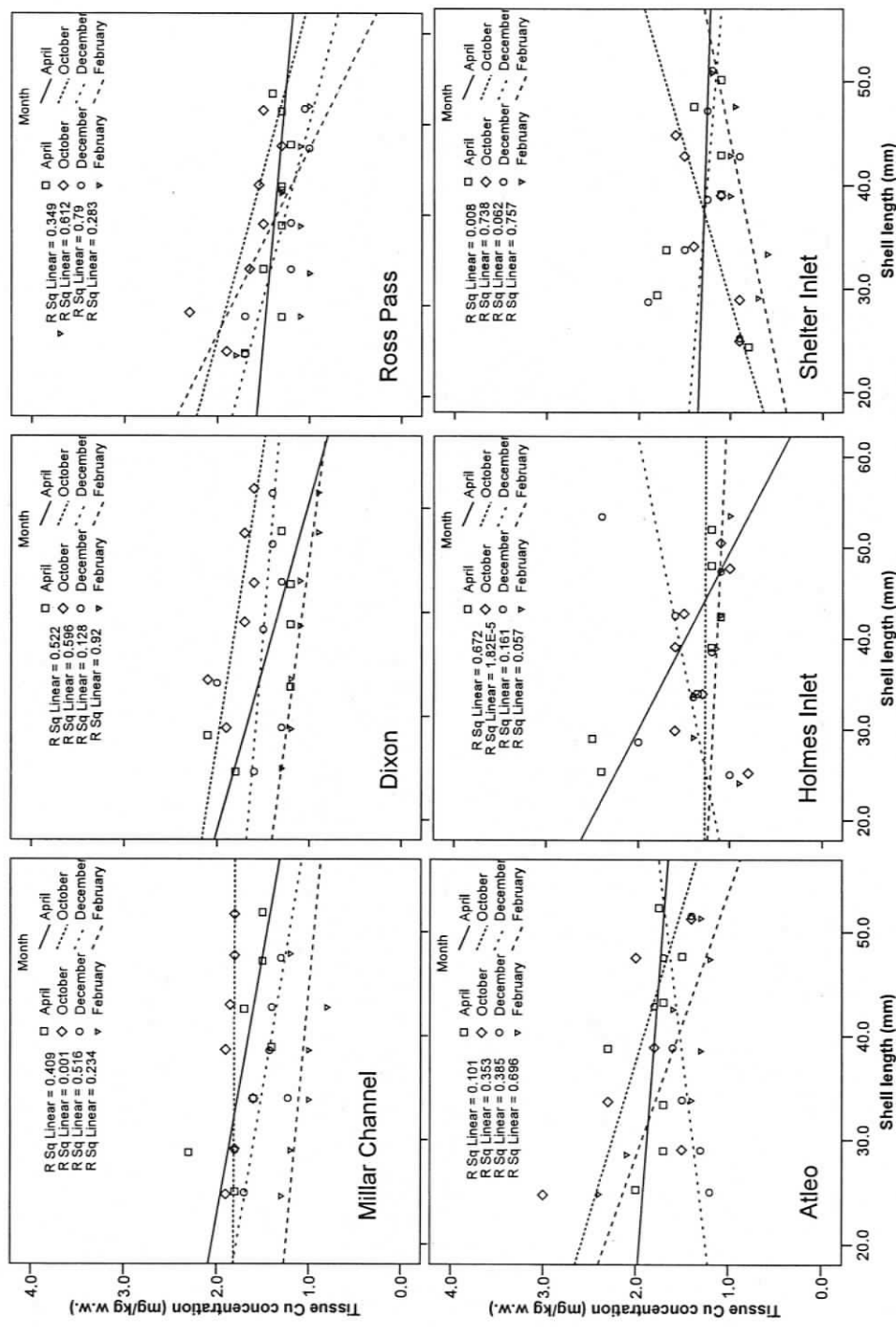
Appendix B. Seasonal differences in metal concentrations in Manila clams



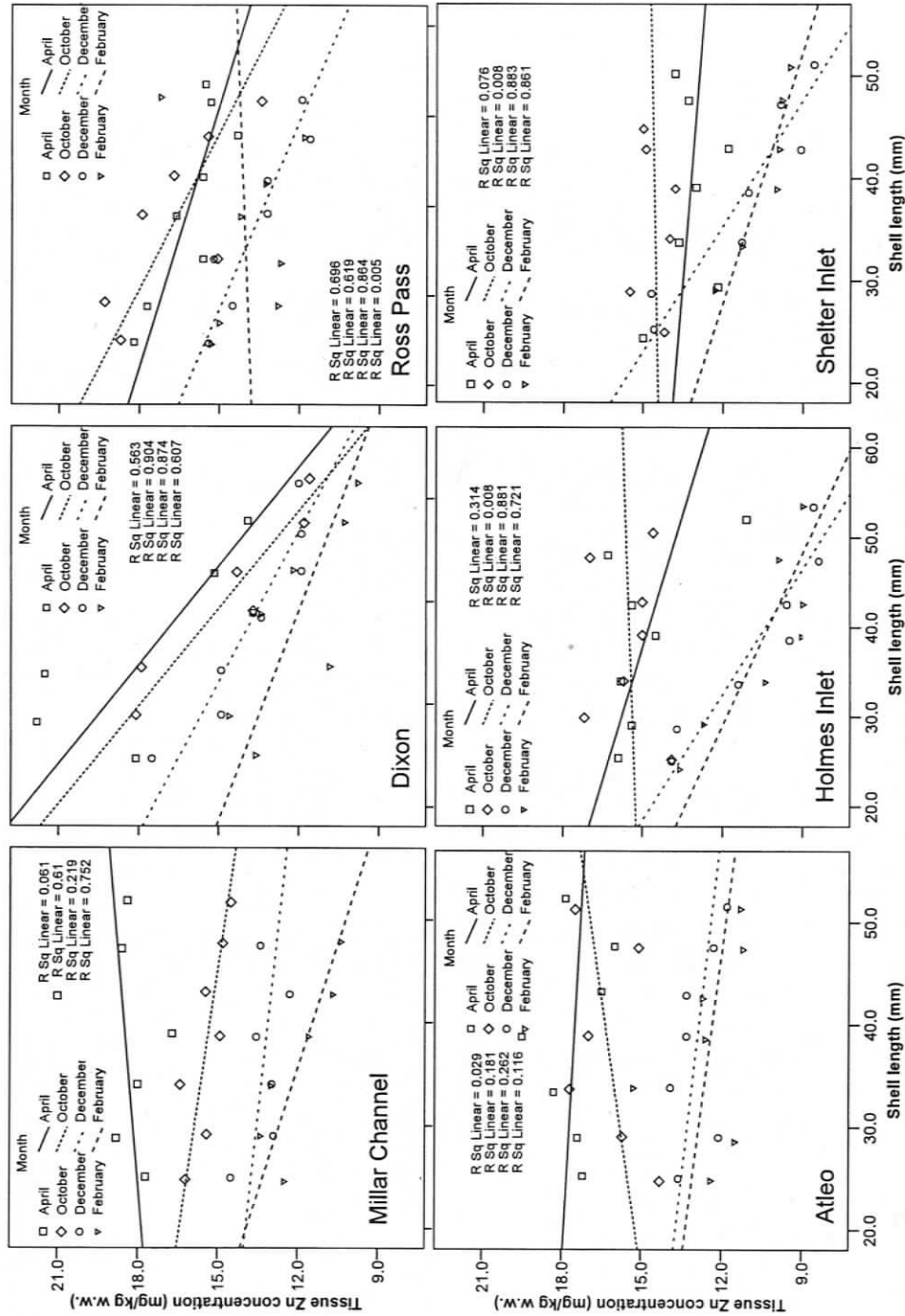
Appendix B1. Arsenic concentrations in Manila clams of increasing shell length in April, October, and December, 2005 and February, 2006. Clams were collected from three sites near salmon farms and three reference sites. The top row depicts the results from farm sites and the bottom row depicts the results from reference sites. The horizontal grey line provides a benchmark for intrasite comparisons.



Appendix B2. Cadmium concentrations in Manila clams of increasing shell length in April, October, and December, 2005 and February, 2006. Clams were collected from three sites near salmon farms and three reference sites. The top row depicts the results from farm sites and the bottom row depicts the results from reference sites. The horizontal grey line provides a benchmark for intrasite comparisons. Note- data are the inverse of the cadmium concentration in tissue and are in the reverse rank order.

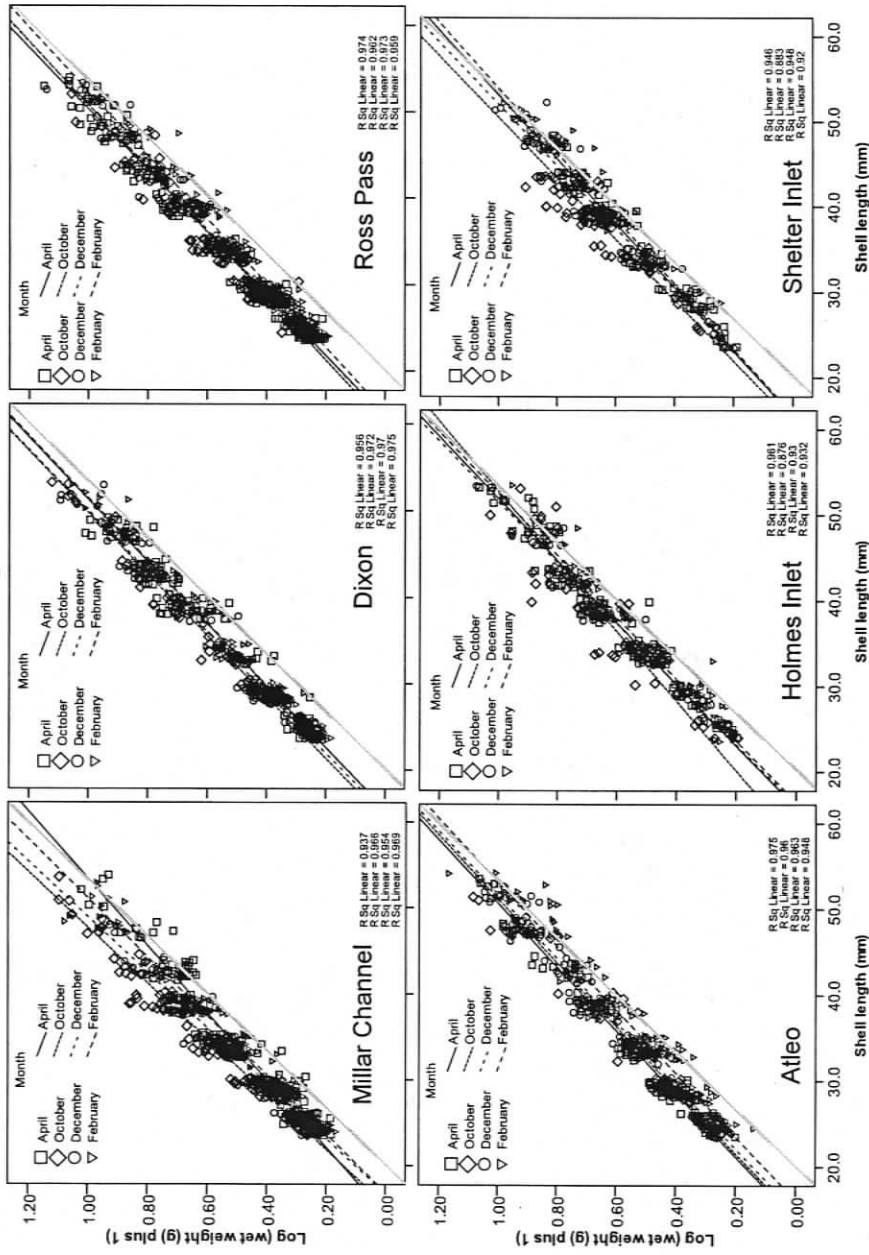


Appendix B3. Copper concentrations in Manila clams of increasing shell length in April, October, and December, 2005 and February, 2006. Clams were collected from three sites near salmon farms and three reference sites. The top row depicts the results from farm sites and the bottom row depicts the results from reference sites.



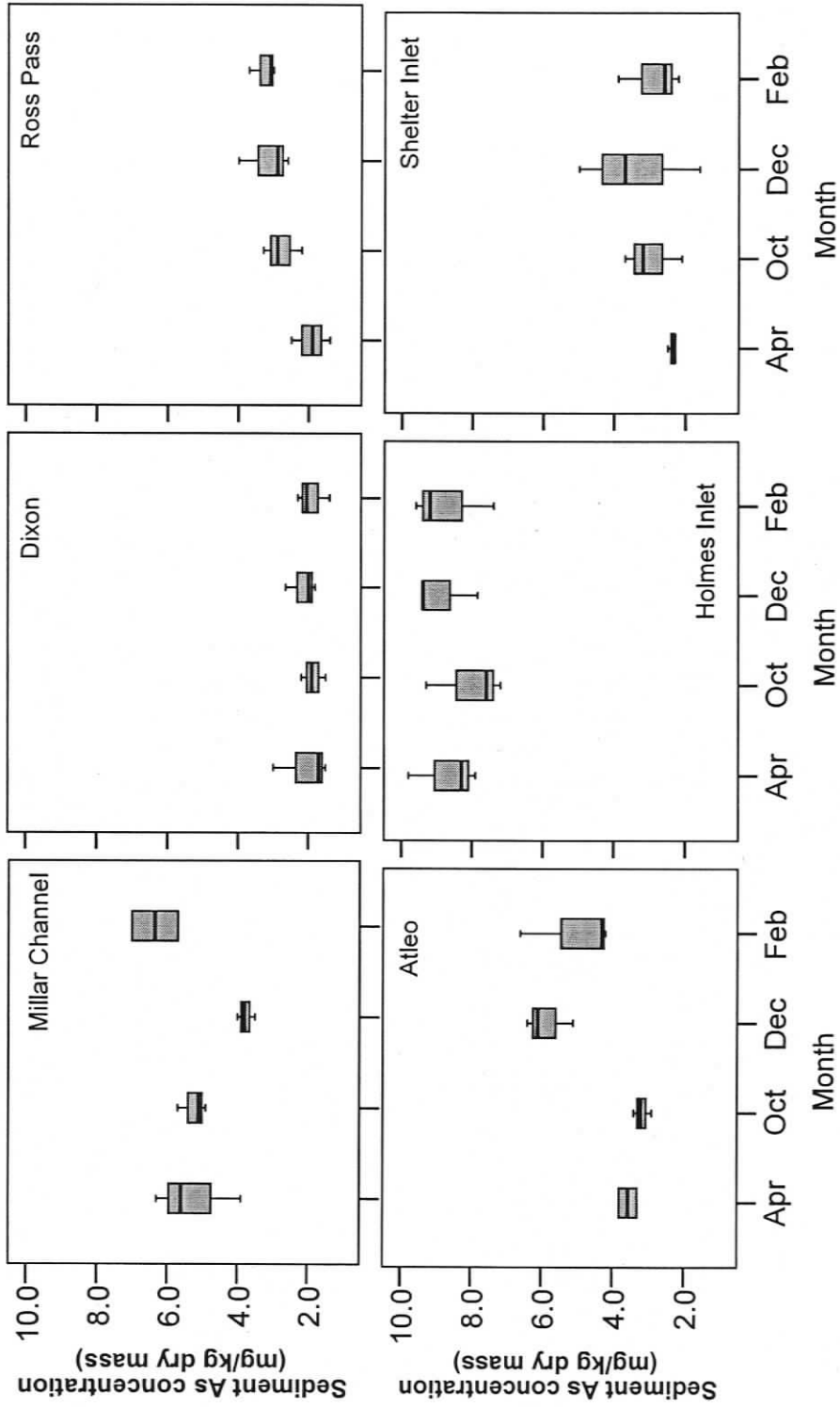
Appendix B4. Zinc concentrations in Manila clams of increasing shell length in April, October, and December, 2005 and February, 2006. Clams were collected from three sites near salmon farms and three reference sites. The top row depicts the results from farm sites and the bottom row depicts the results from reference sites.

Appendix C. Seasonal differences in tissue weight of Manila clams.

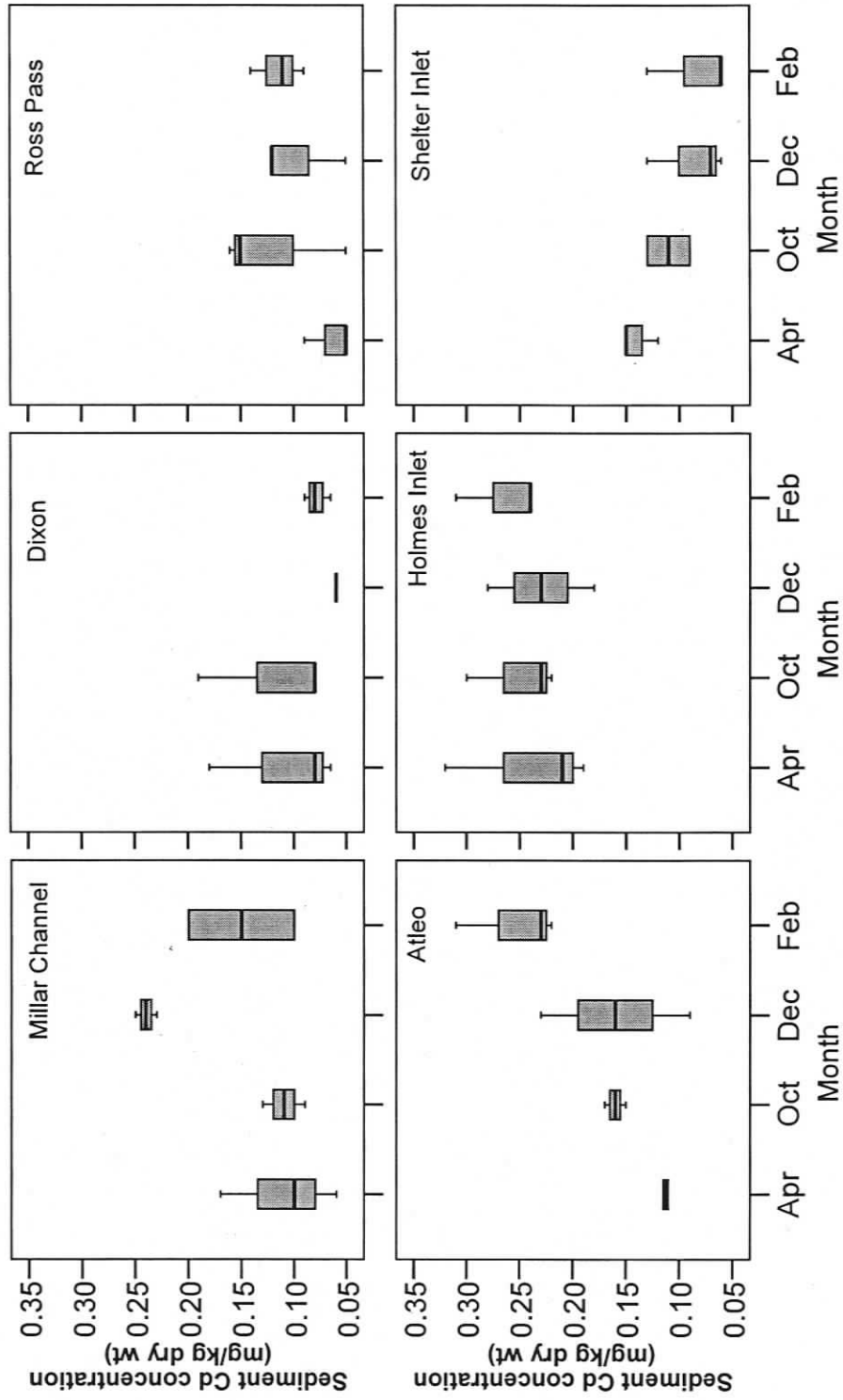


Appendix C1. Tissue wet weight (g) in Manila clams of increasing shell length in April, October, and December, 2005 and February, 2006. Clams were collected from three sites near salmon farms and three reference sites. The top row depicts the results from farm sites and the bottom row depicts the results from reference sites. The horizontal grey line provides a benchmark for intrasite comparisons.

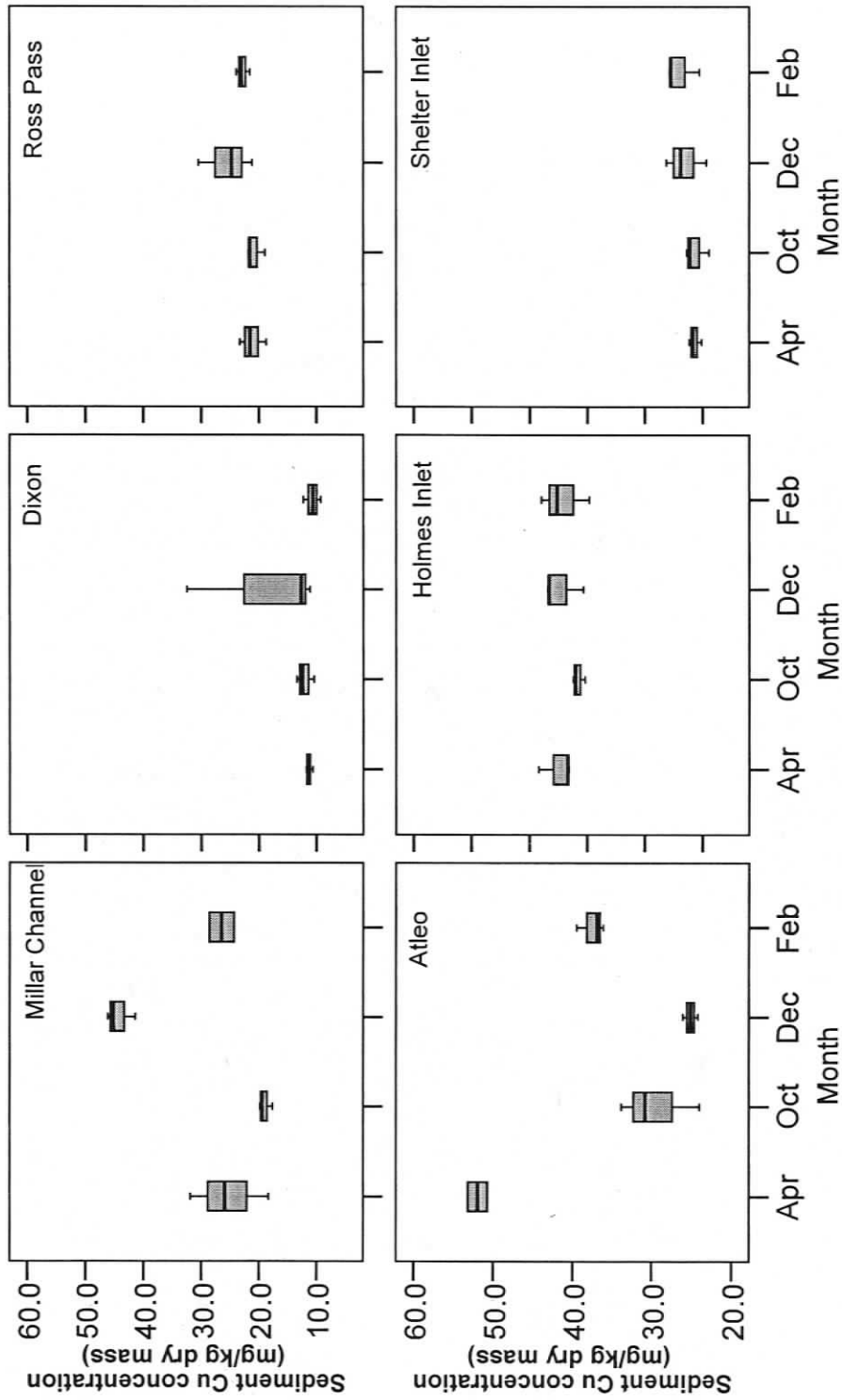
Appendix D. Seasonal differences in metal concentrations in sediment.



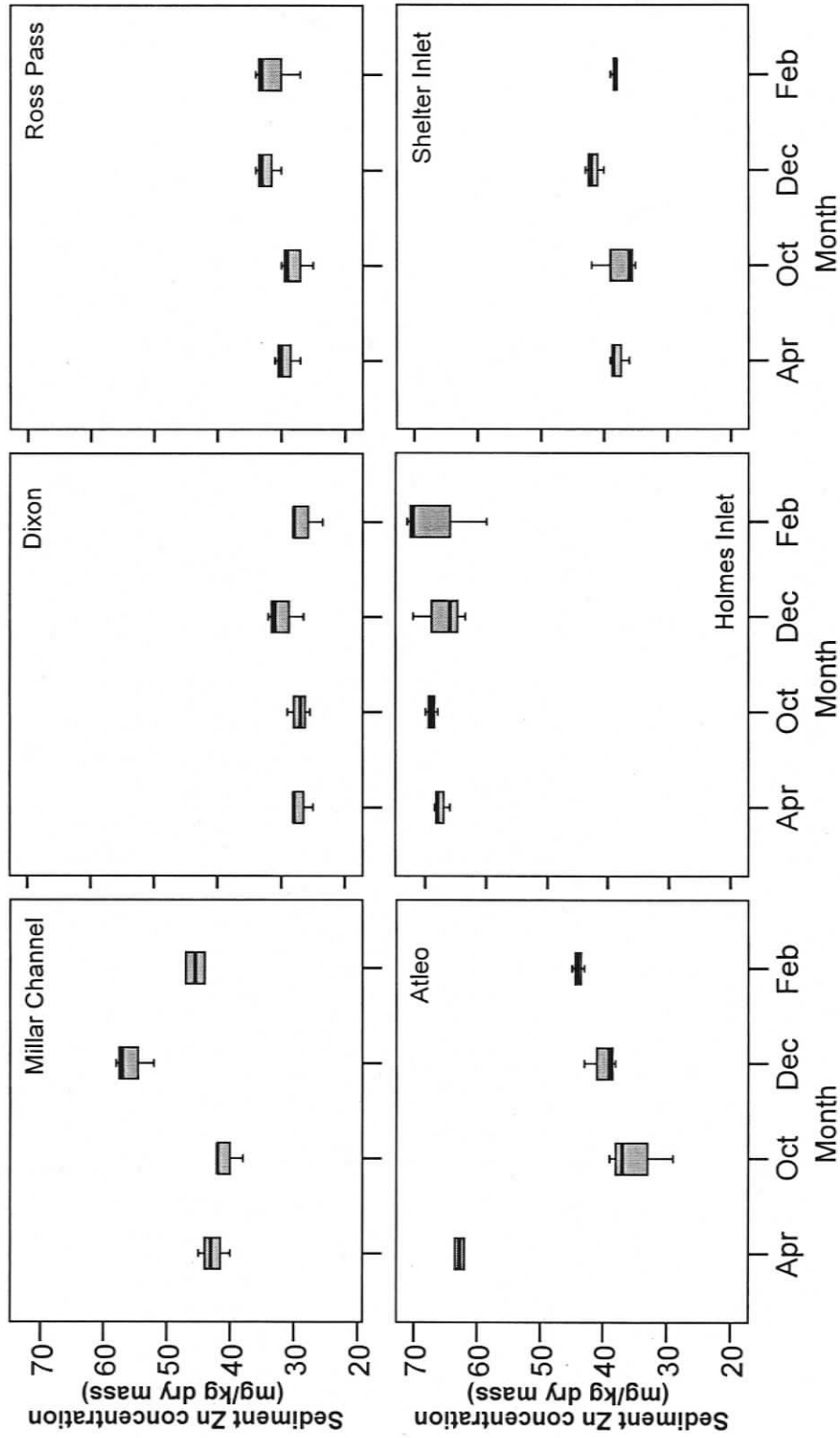
Appendix D1. Arsenic concentrations in sediment collected from three sites near salmon farms and three reference sites in April, October, and December, 2005 and February, 2006. The top row depicts the results from farm sites and the bottom row depicts the results from reference sites.



Appendix D2. Cadmium concentrations in sediment collected from three sites near salmon farms and three reference sites in April, October, and December, 2005 and February, 2006. The top row depicts the results from farm sites and the bottom row depicts the results from reference sites.

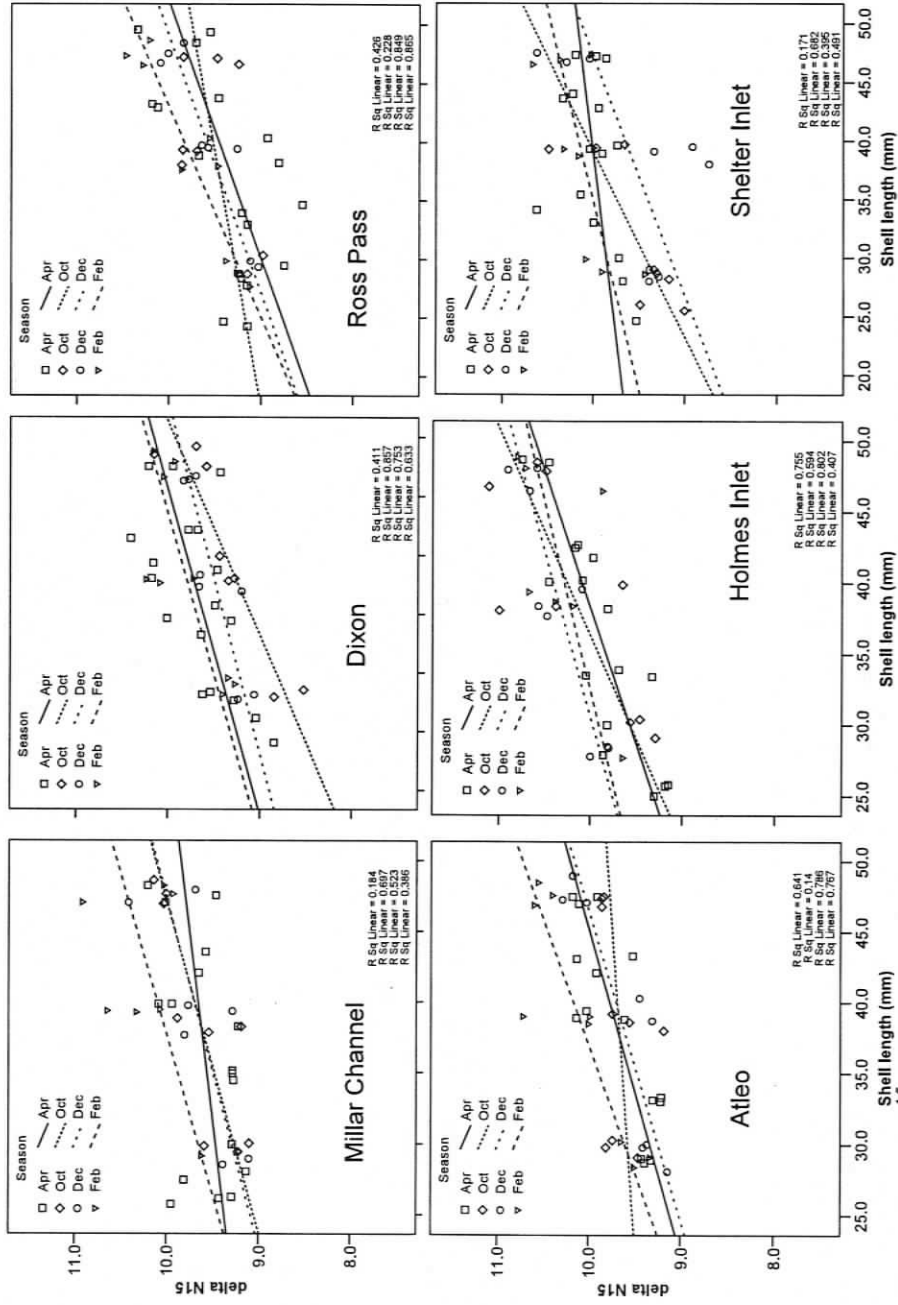


Appendix D3. Copper concentrations in sediment collected from three sites near salmon farms and three reference sites in April, October, and December, 2005 and February, 2006. The top row depicts the results from farm sites and the bottom row depicts the results from reference sites.

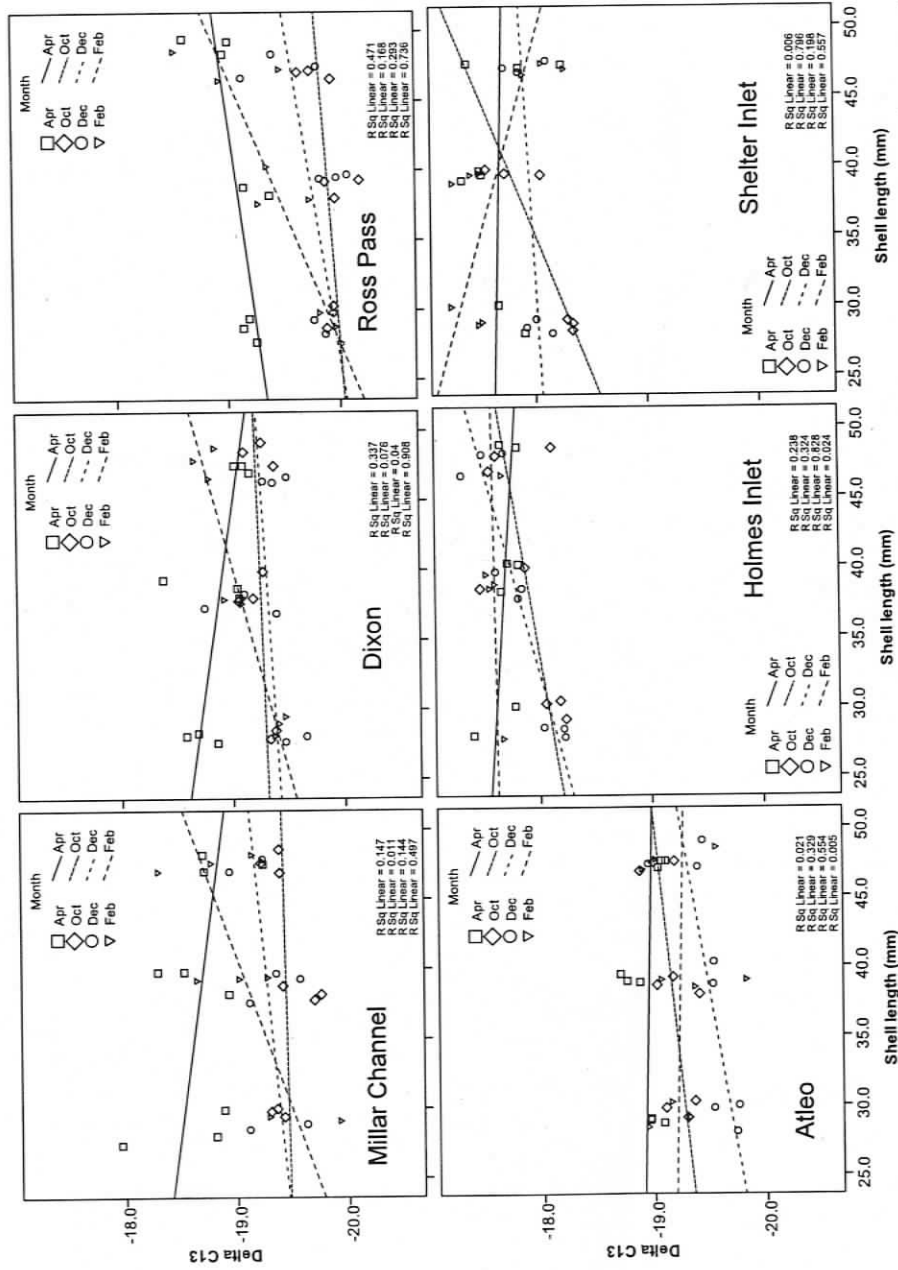


Appendix D4. Zinc concentrations in sediment collected from three sites near salmon farms and three reference sites in April, October, and December, 2005 and February, 2006. The top row depicts the results from farm sites and the bottom row depicts the results from reference sites.

Appendix E. Seasonal differences in stable isotopes of nitrogen and carbon in Manila clams.



Appendix E1. $\delta^{15}\text{N}$ in Manila clams of different lengths collected from three sites near salmon farms and three reference sites in Clayoquot Sound, BC, in four different sampling months. The top row depicts the results from farm sites and the bottom row depicts the results from reference sites.



Appendix E2. $\delta^{13}C$ in Manila clams of different lengths collected from three sites near salmon farms and three reference sites in Clayoquot Sound, BC, in four different sampling months. The top row depicts the results from farm sites and the bottom row depicts the results from reference sites.