Spatial and temporal influence of integrated multi-trophic aquaculture-derived organic effluent on the diet of cultured Pacific oysters (*Crassostrea gigas*), determined through stable isotope analysis.

by

Sarah Jeanine Sprague
B.A., University of Michigan, 2009

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Supervisory Committee

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Abstract

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The goal of this thesis was to detail the spatial and temporal influence of integrated multi-trophic aquaculture-derived organic effluent on the diet of cultured organic extractive organisms within an integrated multi-trophic aquaculture (IMTA) facility in Kyuquot Sound, British Columbia. Naturally occurring and aquaculture-derived sources of nutrients were defined using isotopic analysis of δ15N and δ13C values, in order to examine feeding patterns of a cultured bivalve species *C. gigas*. By examining the diet of *C. gigas* located within the IMTA system, and at a reference site, spatial and temporal patterns of organic-effluent influence on organic extractive components within the IMTA system can be defined. Measurements were performed over four seasons (Spring, Summer, Autumn, and Winter) at a reference site and at stations adjacent to the fish component of the IMTA system, at distances of 0m, 15m, and 30m. Oysters at each station were suspended in the water column at depths of 6m and 18m.

Spatial findings of this thesis focus on the vertical and horizontal patterns of aquaculture derived particulate waste influence on organic extractive components within the IMTA system. Examination of δ15N and δ13C values sampled from *C. gigas* tissue at each station indicated both horizontal and vertical dispersion, and subsequent uptake, of aquaculture-derived effluent. The importance of aquaculture-derived effluent to the diet of *C. gigas* was not uniform across stations or seasons. General trends indicate the strongest reliance upon aquaculture-derived effluent as a nutritional subsidy by *C. gigas* suspended at depths of 18m, and those located 15m and 30m adjacent to the fish component of the IMTA system in particular.

C. gigas feeding patterns analyzed using stable isotope analysis over a temporal scale show seasonal variability in the importance of aquaculture-derived effluent to the diet of organic extractive components within an IMTA system. Examination of δ15N and δ13C values sampled from *C. gigas* tissue at each station indicated strong seasonal shifts

in importance of aquaculture-derived effluent as a nutritional subsidy to oysters within the IMTA system. General trends suggest that *C. gigas* cultured within the IMTA system feed most heavily upon aquaculture-derived effluent during the winter and spring months, while importance of naturally occurring food sources are more heavily relied upon during the summer and fall months.

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1.1 Research Context

Global production of farmed fish and shellfish has more than doubled in the past 15 years. With an average annual growth rate of 6.9% over the past several decades, the aquaculture industry is quickly expanding on a global scale (FAO, 2009). This trend has the potential to relieve pressure on the ocean's fisheries, but the opposite can be true for some types of aquaculture. Current practices of intensive monoculture fish farming have been shown to have a wide variety of negative impacts on the surrounding environment.

Farming carnivorous fish species requires large inputs of wild fish for feed. Some aquaculture systems also reduce wild fish supplies through habitat modification, wild seed stock collection and other ecological impacts (Silva et. al., 2012). Harmful effects to the surrounding marine environment include the large amounts of organic waste and inorganic nutrients coming from uneaten feeds and from feces/excretions of cultured fish.

Excess nutrients introduced into aquatic ecosystems, which are often nutrient-limited, have been shown to cause eutrophication, harmful algal blooms, reduction in levels of dissolved oxygen, and changes in the microbial composition of benthic community (Ozbay, G., 2008). Aquaculture waste is high in nitrogen and phosphorus concentrations dissolved from waste feed and fish feces, which has been shown to have harmful effects on the surrounding environment (Iwama, K., 1991).

In addition to the harmful ecological effects of large-scale monoculture systems, negative social and economic impacts include the loss of natural goods and services, suggesting the need for changes within the industry (Flaherty, M. 2009). However, despite the negative impacts, gains from many aquaculture industries outweigh those from the wild caught fisheries (Naylor, L. 2000). If this rapidly expanding industry is to sustain its contribution to world fish supplies, it must reduce wild fish inputs in feed as well as waste input to marine ecosystems, by adopting more ecologically sound management practices.

There is a need for more efficient cultivation methods worldwide, including the development of integrated systems that are based on ecological engineering principals, and are established to mirror natural ecosystems, thus requiring less resource input, while minimizing waste. Integrated multi-trophic aquaculture (IMTA) has received much attention as a bioremediation method that can be used to mitigate negative environmental

impacts and maintain the sustainability of aquaculture systems (Silva et al., 2012). This is a variation of an historic practice of aquatic polyculture, which is the co-culture of different fish species from the same trophic level. In this case, farmed organisms may all share the same biological and chemical processes, with few synergistic benefits, which could potentially lead to significant shifts in the ecosystem.

Some traditional polyculture systems can, in fact, incorporate a greater diversity of species, occupying several niches, as extensive cultures (low intensity, low management) within the same pond. The integrated aspect of IMTA involves the more intensive cultivation of different species in proximity to each other, connected by nutrient and energy transfer through water (Robinson, S. 2009). A complex system, IMTA attempts to address the dispersal of organic wastes produced by many fish farms, through a series of engineered biotic processes. Finfish farming in open ocean settings may result in a high loss of organic matter to the surrounding environment, in the form of faeces and uneaten feed. The dispersion of faecal matter and feed particles is highly dependent on the local hydrodynamic regime, among other environmental factors.

IMTA is a form of ecological engineering that combines the biological processes of cultured fish and extractive co-cultured species to remove waste loadings associated with intensive aquaculture systems (Chopin et. al., 2001). This approach takes into consideration site specificity, operational limits, and food safety guidelines and regulations. The goals are to achieve environmental sustainability through biomitigation, economic stability through product diversification and risk reduction, and social acceptability through better management practices. Multi-trophic refers to the incorporation of species from different trophic or nutritional levels in the same system (Chopin and Robinson, 2004).

Co-culturing practices place macroalgae and suspension or deposit feeders in the role of biofilters, which reduce the amount of aquaculture-derived inorganic nutrients and particulate organic waste. In this context, the success of IMTA depends upon the effectiveness of the extractive co-cultured species to absorb fish farm waste. To evaluate the effectiveness of an IMTA system, it is necessary to assess whether the extractive species co-cultured with the target fish assimilate the aquaculture-derived waste within the system. Knowledge about the transfer and uptake of waste materials from the farmed

fish to co-cultured extractive species in the IMTA systems has advanced in recent years (Jan et al., 2014, Parks et. al., 2015). This thesis attempts to further develop this body of study.

In several jurisdictions, open-water aquaculture is regulated through measures of benthic hydrogen sulfide concentrations, which is proportional to excess deposition of organic material such as feces and uneaten food precipitating from farmed fish cages. Interception, consumption, and digestion of organic portions from the fed trophic level by organic extractive species results in ‘‘organic stripping,’’ with less organic material in resulting feces, thereby reducing the net organic load and benthic deposition potential.

Shellfish are deployed beside fish cages in some open water IMTA systems. In addition to the potential consumption of fish culture solids, natural particles (seston) are also consumed. Consumption of seston by shellfish means that some suspended solids that would otherwise drift by fish cages now have the potential for redirection to the benthos as indigestible seston components, egested in shellfish feces. This raises the issue as to what dietary proportion of fish culture solids, consumed by extractive species, results in an increase or reduction of net organic load.

IMTA permits intensive growth and harvest of co-cultured species that represent separate trophic levels within the same system, where allochthonous nutrient effluent subsidizes each group through water currents and gravity (Neori et al. 2004). Within these systems, uneaten fish feed and fish feces are transferred to other cultured species as usable nutrient inputs. Therefore, these additionally placed organic and inorganic extractive species are also harvestable and represent a source of economic revenue (Reid et al. 2008). Significant increases in growth of organic extractive bivalves and inorganic extractive kelp (*Saccharina latissima*) reared adjacent to Atlantic salmon (*Salmo salar*) have been measured within an IMTA facility in the Bay of Fundy, Canada (Reid et al. 2008) compared growth of mussels *Mytilus galloprovincialis* both reared adjacent to and 1000 meters upstream from fish cages and determined IMTA site mussels were characterized by greater total length, biomass, and weight than those sampled far from cages.

IMTA systems are designed to harness the energy stored in these waste materials through the use of filter feeding bivalves, as well as kelp species, which incorporate the

potentially environmentally harmful nutrients into biomass, representing a valuable commodity in the seafood industry. In particular, Qualicum Bay Scallops and Pacific Oysters are marketable products in British Columbia, and can be farmed alongside various species of finfish as both environmental buffers and economically lucrative commodities.

1.2 Research Focus.

In Kyuquot Sound, one farm site serves as an example through which the mechanics of IMTA systems can be explored. The system is comprised of a fed component which is raised in tandem alongside extractive components. In order for extractive components to be as effective as possible in the uptake of waste, the relationship between the spatial and temporal patterns of waste produced, and the extractive components' effectiveness under a variety of possible conditions, must be understood. Stable isotopes, specifically stable Nitrogen and Carbon species, have been shown as effective tools in measuring flow of nutrients through biotic interactions (Peterson, 1999; Vizzini and Mazzola, 2004). In this instance, stable isotopes may be used to determine the ideal placement of extractive components within an IMTA system, by measuring isotope ratio levels present within the extractive species when placed at specific depths and distances, surrounding the fed component. By determining ideal placement for scallops and oysters within IMTA systems, maximum efficiency in both environmental and economic services can be attained.

The collective body of literature examining potential benefits of IMTA systems, the use of stable isotopes as tools for tracking nutrient distribution through aquatic systems, and the design of IMTA systems is immense. IMTA systems are specialized forms of polyculture, engineered to be environmentally benign through the use of organisms at multiple trophic levels (Chopin, 2010). Because the farm site in Kyuquot Sound exists in an open-water coastal setting, the system design must inherently address a diverse set of variables when considering where and how to install each component. Because IMTA systems in a coastal setting are subject to seasonal fluctuations in current strength and direction, temperature, and fluctuations related to each organism within the system, a clear picture must be developed of how these components interact, in order for

the system to operate at maximum efficiency (Troell, 2009). It has been shown that fishes in aquatic systems, regardless of the practice of aquaculture, consume stable isotopes in their diets, thereby introducing these isotopes into the system itself (Schroeder, 1983).

Because stable isotopic ratios can be tracked through multiple trophic levels, they can be used as a tool to map the flow of nutrients through an aquatic system (Bennett, 2006). Carbon 13 and Nitrogen 15 isotopes in particular are present in fecal matter of fishes, as well as the tissue of extractive components such as oysters and scallops (Reid, 2008), and are therefore an appropriate measure of fecal matter distribution within an aquatic system. Because the goal of IMTA systems is to situate extractive components for maximum uptake of fish fecal matter, it is imperative that the relationship between efficiency of extractive components in a variety of spatial locations and densities, in relation to the fed component, is understood.

Marine food webs and primary productivity are generally determined by multidirectional transport of nutrient and detrital materials. Transport can occur both vertically, through upwelling and detrital sinking, and horizontally, through currents, tidal motion, and eddy diffusion (Polis et al., 1997). Daily migrations by pelagic fish and zooplankton rapidly transport nutrients across habitats. These nutrients include fecal matter rich in fertilizing nitrogen useful to bottom dwelling detritivore communities. Dissolved ammonia and other inorganic compounds found in fish feces promote growth in phytoplankton and macrophytes throughout the photic zone of the water column (Coen & Neori, 1991). Primary producers are essential for secondary production, and therefore mark nutrient subsidies as being vital in shaping diversity, density and biomass of species within food webs (Vizzini & Mazzola, 2003). Overproduction of phytoplankton due to overabundant concentrations of particulate organic and inorganic matter by- products can stimulate conditions that consequently lead to anoxia and eutrophication (Troell & Berg, 1997).

Pacific oysters (*Crassostrea gigas*) are a common inhabitant of coastal British Columbia, being found throughout marine habitat spanning the entire coast, and are considered a potential candidate for biomarkers of industrial waste (Julshamn and Grahl-Nielsen, 1996). This relationship may extend to salmon farm waste in the form of fecal matter and fish feed. Qualicum Bay Scallops are currently used in this context through

the integration of bivalve mollusks within fish culture operations, where they are touted as a potential method of efficiently reducing the environmental impact of organic waste from fish farms (Soto and Mena, 1999).

Previous studies have determined that Oysters can be successfully incorporated into IMTA systems with finfish (Lander et al., 2004) based on growth of extractive components to marketable size. Whether fish farm waste contributes to the success of such integrated systems has not been fully quantified, and a causal link between observed increased growth rate, and proximity to finfish nets, has not been established. It is therefore necessary to develop methods to determine if organisms, both in an integrated system and in the wild, are consuming waste fish feed. By developing and employing these methods, organic extractive components could then be carefully placed within a system to maximize efficiency.

Several methods have been developed to measure the transfer of energy through consumer-resource communities. These include direct field and laboratory observations of predator-prey interactions (Duggins, 1980), stomach content analysis (Marion et al., 2008), radio-tracer techniques (Wang & Fisher, 1999) and natural stable isotopes analyses (Schindler & Lubetkin, 2004). Depending on the food web being analyzed, field observational studies tend to be inconclusive as sufficient sample sizes are difficult to obtain.

The drawbacks to laboratory studies include restrictions in time and space, as well as an artificial in vitro environment, which can potentially challenge the ability of a researcher to obtain practical results. Gut content analyses can be time consuming, and require extensive specimen collection and dissection of content that will likely represent only a snapshot of a consumer diet (Schindler & Lubetkin, 2004). In addition to these limitations, organisms digest their prey at varying rates, which can bias results as content becomes less intact or recognizable over time (Michener & Schell, 1994). Radio-tracer techniques have been used by artificially and uniformly labeling a potential prey or compound with an isotope or dye, then releasing the labeled organism, measuring the uptake or loss of the isotope upon retrieval (Conover & Francis, 1973). Once all labeled specimens are retrieved, this method can be reasonably conclusive. However, recovery of a statistically significant number of labeled specimens can be difficult logistically.

Stable isotope analysis applied to food web dynamics questions are widely used, because of the potential for isotope data to provide both source-sink and process information through the use of a mass-balance approach applied across trophic levels (Peterson & Fry 1987).

Ecosystems are often depicted in the form of a food web, where predator and prey species occupy variable positions in trophic niche space and time linked through energy transfers (Paine, 1980). Early models placed species into discrete trophic levels, where primary producers occupy basal levels that increase linearly towards secondary consumer species. Within this idealized trophic structure, trophic levels are determined by the number of times chemical energy is assimilated, or transformed, from a consumer‘s diet into its biomass (Oksanen *et al*., 1981).

However, food webs have since been shown to exhibit highly complex and spatially heterogenous relationships. Current models contain thousands of species connected via multiple and variably strengthened linkages with consumption and productivity spatially existing in a number of directions throughout the community (Polis *et al*., 1997). Linear models are, unable to adequately accommodate the complex dynamics of detritus, omnivory, spatial resource subsidies across habitats, looping and/or nutrients. Despite oversimplification for ease of trophic depictions, the classic food chain model continues to provide the basis for most food web studies today (Dunne *et al*., 2004).

Data acquisition for stable isotope analysis is straightforward, and analysis is done through the sampling of a food web subpopulation by advanced analytical technology. Stable isotope analysis has been used to determine sources of nutrition for consumers, to study food web dynamics across trophic levels, and to trace waste products from aquaculture (Mazzola, 2004). The use of stable isotopes as tracers relies on the food source and the consumer having distinct signatures.

The application of stable isotopes analysis has been widely used to trace the influence of nutrient loading within tissues of marine communities adjacent to both mono- and polyculture facilities (Dubois et al. 2007). Gao et al. (2006) used the carbon and nitrogen stable isotope approach and determined enrichment of signatures among bivalve communities reared adjacent to fish cages as compared to those sampled at a

reference site. This indicated the uptake and assimilation of isotopically heavier fish feed and fish faeces. Ruiz et al. (2010) measured significantly elevated δ15N signatures within epiphyte and seagrass leaf tissues adjacent to a large fish farm facility. Currently, there is a dearth of research that has utilized stable isotope analysis to describe trophic linkages between extractive species within an IMTA facility.

Stable isotope ratios from food sources are incorporated into animal tissues following assimilation (Pinnegar and Polunin, 1999). Pacific Scallops have been shown to respond to a switch in diet in approximately 20 days for both δ 13C and δ15N, with ratios varying depending on age and tissue sampled (Dubois et al., 2007). Differences in tissue response times have been shown for other bivalve species (Paulet et al., 2006). Generally, there is enrichment of the heavier isotope forms (13C, 15N) as the lighter isotopes (12C, 14N) are preferentially digested during metabolism (Dubois et al., 2007). The assimilation of nutrients can therefore be measured by a shift in the stable isotope signature in the direction of the food source (Paulet et al., 2006).

In tandem, stable carbon (13C) and nitrogen (15N) isotopes have been used to trace the destination of fish farm waste including uptake in adjacent bivalve communities (Dolenec et al., 2007). It has been proposed that when ambiguity in tracing signature exists, dual tracers may help identify the true food source (Gao et al., 2006) with some studies using both 13C and 15N stable isotopes to trace food sources and diet (Xu and Yang, 2007).

In order to measure assimilation over varying temporal scales, it is important to note that different tissues within an individual exhibit different isotopic values, with varying rates of tissue turnover. This turnover is attributable to growth and metabolic tissue replacement. If organisms are selectively feeding, isotopic values alter over time to match those of their new diets, and different tissues within the organism will alter at different rates (Michener & Schell 1994). Studies have demonstrated that more metabolically active tissues reflect this change more quickly (Layman et al., 2012). Unless the study organism is an indiscriminant feeder, selecting tissues with higher turnover rates for isotopic analysis would be ideal in order to get a picture of diet over time (Kang *et al*., 1999). Often the diets of consumers vary over time, and this is not immediately manifested in its isotopic signature (Boeckle et. al., 2011). There is often a

lag time. Cheung, S.G., (2006) analyzed seasonal variation in isotopic compositions within a marine food web and found a general depletion of values in winter and enrichment of values in summer. He sampled fish muscle tissue, and found that this tissue responded relatively quickly to dietary changes, and was generally less variable than in other organs.

It has been demonstrated that leftover fish feed generated by an aquaculture facility was incorporated into the whole body tissues of the bivalve communities using both the 13C and 15N stable isotopes. Lander et al. (2004) showed that bivalves are suitable for integrated multi-trophic aquaculture with Atlantic salmon, which are finfish similar in trophic niche to that of Sablefish. In addition, mussels cultured at various salmon farm stations showed increased growth rates and reached a significantly larger size than those in naturally occurring locations (Lander et al., 2004). By analyzing food web dynamics within an IMTA system, using stable isotope analysis, optimal placement of extractive components can be identified and employed. By examining nutrient uptake efficiency of Pacific Oysters at varying distances from finfish nets, maximum utility of an IMTA system may be achieved, reducing environmental impact and increasing economic viability of the operation itself.

Isotopic compositions are formally expressed as parts per thousand (‰) differences, as δ-notated values, from the formula (Peterson & Fry 1987):

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 103$$

where δ is the sample isotopic measurement notation (heavy to light atomic weight ratios), X equals 13C and 15N, and R corresponds to the ratios of 13C/12C and 15N/14N. Changes in the δ value reflect ratio differences in heavy to light isotopic values. For instance, as δ15N increases in a sample, the proportion of 15N (heavy) increases, while the proportion of 14N (light) decreases (Peterson & Fry, 1987). δ values are then referenced to known standard materials, depending on the isotope analyzed (represented by R standard in the above equation). For instance, carbon is compared to PeeDee limestone and nitrogen to atmospheric nitrogen gas (Peterson & Fry, 1987). A dual or triple collector isotope ratio mass spectrometer measures these values in a sample's gaseous form; dissected sample tissues must first be ground to a powder and converted to pure gas (CO2 and N2). The resulting sample data can be compared to predicted sampled

source values from a food web under study.

Despite the similarity in the chemistry of heavy and light isotopes, their hydrodynamic properties differ slightly due to different atomic masses (Schindler & Lubetkin, 2004). This difference leads to variable rates of biochemical reactions resulting in biofractionation, or, the change in isotopic ratio within an organism in relation to its diet. The process of biofractionation is variable for each isotope. For carbon, it begins with photosynthesis and can be traced all the way to the tissues of secondary consumers. Carbon and sulfur δ values obtained from individual animals will be representative of their diets, with an expected slight enrichment of approximately 0.5 to 1 ‰ (Michener & Schell, 1994).

Carbon is widely used to provide source information of primary production from the surrounding environment and can be used to analyze direction of flow from primary producers to consumers. As for nitrogen, measured δ values are heavier than their dietary counterparts, at approximately 3 to 4 ‰ enrichment relative to prey (Frederiksen, 2003). The 15N enrichments in animal isotopic versus dietary composition are mainly due to the excretion of 14N in urine, which is 15N depleted. Nitrogen isotopic ratio values for feces are congruent with those of the animal composition (Peterson & Fry, 1987), which is thus a powerful tool for identifying trophic positions of organisms within a food web (Vizzina & Mazzola, 2002).

Nitrogen is the limiting nutrient in the majority of marine environments (Becker, K. 1998). On the west coast of North America, relatively cold marine waters are especially productive and often high in nitrate, which is an important source of nitrogen to the euphotic zone. The Coriolis effect causes these nutrient-rich waters to be replaced by surface waters, as they are pushed offshore by northwesterly winds. A large proportion of phytoplankton are carried below the photic zone by wind-driven vertical mixing, where photosynthesis cannot be carried out. For this reason, light penetration through water is highly limiting in high latitudinal regions such as British Columbia, further repressed by onshore winds that cause thick and constant cloud cover in most of this region.

Limited light occurs over relatively long temporal periods and particularly in winter months. These factors result in primary production often being largely more light-

limited than nutrient-limited within coastal Pacific Northwest waters (Horner *et al*. 1997).

1.3 Research Objectives:

This research examines an IMTA facility located in Kyuquot Sound in British Columbia, in order to better understand the importance of aquaculture waste to the diet of extractive species on a spatial and temporal basis. The purpose of this thesis is to measure the degree to which integrated multi-trophic aquaculture (IMTA)-derived organic waste acts as a source of nutrients for extractive organisms.

Heavy particles are subject to rapid sedimentation, while less dense or finer particles are likely to be carried some distance from a fish farm. The distance that these particles are transported determines placement of intended extractive components within the IMTA system (Kutti et al., 2007). The influence of aquaculture-derived organic waste on the diets of intended extractive organisms can be measured through stable isotope analysis. This thesis attempts to answer some essential questions pertaining to the uptake of aquaculture-derived waste by intended extractive components on a spatial and temporal scale. The goals of this thesis are to:

1. Establish an isotopic baseline for a Kyuquot Sound IMTA system and surrounding pelagic environment.
2. Identify spatial variability in importance of IMTA-derived organic effluent to the diet of Pacific oysters cultured within an IMTA system.
3. Identify temporal variability in importance of IMTA-derived organic effluent to the diet of Pacific oysters cultured within an IMTA system.

In order to meet these objectives, I measured the δ13C and δ15N stable isotope compositions of intended extractive organisms at a reference site and within an IMTA facility located in Kyuquot Sound, British Columbia every three months over the course of one year.

In Chapter two, I compare carbon and nitrogen stable isotope signatures of Pacific Oysters within the IMTA system, in relation to their distance from the fed component and

depth in the water column. I examine temporal variation in contribution of IMTA derived effluent to the diet of oysters within the IMTA system as well, in order to look at seasonal trends in filtration ability.

In Chapter three, I conclude with a summary of the research presented in this thesis, and discuss the possible implications for IMTA system design.

Past studies have focused primarily on far-field effects of nutrient dispersal around aquaculture stations. This study aims to identify the effect of fish net arrays on near-field, down-current nutrient availability to organic extractive components. The results from this study can be used as a guide in the placement of co-culture extractive species at IMTA stations, in order to optimize capture of nutrients released from fish farms. Potential benefits of this study to industry include the demonstration of a direct link between aquaculture waste and its subsequent uptake by filter feeding bivalves on a seasonal basis, as well as providing guidelines for the efficient placement of organic extractive components.

Chapter Two: Analysis of spatial and temporal variability in the importance of integrated multi-trophic aquaculture-derived organic effluent as a nutritional subsidy to the diet of cultured Pacific oysters (*Crassostrea gigas*), using stable isotopes.

2.1 Abstract

Using stable isotope analysis, spatial and temporal patterns of aquaculture derived particulate waste influence on organic extractive components within an IMTA system were examined. Pacific oysters (*Crassostrea gigas*) were suspended in the water column in lantern nets at depths of 6m and 18m at four stations (three within the IMTA system, and one reference) and tissue was sampled and analyzed over the course of one year. Analysis of δ15N and δ13C values sampled from *C. gigas* placed at stations located 0m, 15m, and 30m adjacent to the fed fish (*Anoplopoma fimbria*) component of an IMTA system, at depths of 6m and 18m, indicated both horizontal and vertical dispersion, and subsequent uptake, of aquaculture-derived effluent. Results show significant differences between aquaculture derived waste consumption at different stations within the IMTA system, as well as between the reference site and IMTA system stations. General trends indicate the strongest reliance upon aquaculture-derived effluent as a nutritional subsidy by *C. gigas* suspended at depths of 18m, and those located 15m and 30m adjacent to the fish component of the IMTA system in particular.

General trends also suggest that *C. gigas* cultured within the IMTA system feed most heavily upon aquaculture-derived effluent during the winter and spring months, while importance of naturally occurring sources of nutrition are more heavily relied upon during the summer and fall months.

2.2 Introduction-Spatial

Concerns about environmental impacts of the growing aquaculture industry are rising, as intense monoculture becomes the norm in standard industry practice. Potential issues include the sedimentation of particulate matter, which may cause the organic enrichment of sediments, having a negative effect on the benthic community if sedimentation rates

exceed the turnover rate of the community (Kalantzi and Karakassis, 2006), while dissolved nutrients may cause eutrophication (Skogen et al., 2009). In order to minimize the potentially negative effects of waste discharges, the practice of cultivating extractive and filter feeding species at lower trophic levels in close vicinity to fish farms, a strategy termed IMTA, or integrated multi-trophic aquaculture, is of interest.

IMTA is used as a means of obtaining increased biomass production, thus adding to the value of feed investments, and at the same time contributing to a more sustainable aquaculture production (Troell et al., 2009). The dissolved inorganic nutrient wastes can be taken up by inorganic extractive species such as seaweeds (Buschmann et al., 2001), while released particulate organic nutrients can be consumed by filter feeding species such as mussels and oysters. Several studies have suggested that bivalve filter feeders can provide bioremediative services when co-cultivated with fish aquaculture, hence reducing the environmental impact associated with a great release of particulate organic matter from marine cage aquaculture (Cheshuk et al., 2003).

An effective IMTA operation requires careful selection and placement of various species, in order to capture both particulate and dissolved waste materials generated by fish farms. The selected species and system design should be engineered to optimize the recapture of waste products. As larger organic particles, such as uneaten feed and feces, settle below the cage system, they are eaten by deposit feeders, such as sea cucumbers and sea urchins. At the same time, the fine suspended particles are filtered out of the water column by filter-feeding animals such as mussels, oysters and scallops.

Many studies have focused on the potential capacities of filter feeding organisms to capture and consume waste particles from the water column at varying concentrations and current speeds (Neori et al., 2004). This research aims to look at the importance of farm waste to the diet of filter feeding bivalves, in order to determine where in the system they are most effective at consuming waste particles. Each IMTA system is unique due to varying current regimes, species configurations, and hydrodynamic properties. However, the ability to generalize where in a system filter feeders are most efficient would be useful for overall system design.

IMTA has been in practice in various forms for centuries. Civilizations most successful at developing integrated aquaculture systems treat wastes as valuable resources, and have a long history of integrating nutrient cycling into their agricultural systems (Chiang, 1993). The discipline of ecological engineering addresses and quantifies the processes that are involved with management of wastes as a resource. Such studies consider a variety of complex environmental and social factors, in addition to maximizing short-term profit (Wilks, 1995). Recent advances in IMTA cultivation techniques evolved primarily from ecological engineering experiments examining the intensive culturing of seaweeds and bivalves as biofilters at sewage outflows (Goldman et al., 1974) and aquaculture outflows (Shpigel, 2005). Environmental concerns about the rapid expansion of intensive mariculture systems have also recently led to a renewed interest in IMTA (FAO, 2006).

In the past fifteen years, the integration of seaweeds into marine fish culturing has been examined and studied in Canada, Japan, Chile, New Zealand, Scotland, Norway and the US. The integration of mussels and oysters as biofilters in fish farming has also been studied in a number of countries, including Australia, the US, Canada, France, Chile, Norway, and Spain (Abreu et al., 2009). The recent offshore relocation of many coastal finfish farms in Turkey has also generated interest in IMTA (Turan et al., 2009). Recent reviews on IMTA research include a focus on seaweeds, bivalves, crustaceans, and on integrated cultures.

Determining ideal placement for filter-feeding organisms within an IMTA system is necessary in order to maximize efficiency of environmental services, as well as increase the output of marketable commodities. Placing extractive components in areas where growth rate is maximized will generate an economically lucrative product, as well as provide an important environmental service through maximized filtration. Ideal placement can be achieved by examining hydrodynamic properties in and around the aquaculture site, understanding the capacity of extractive organisms to perform under various conditions, and by understanding a farm's waste dispersal patterns. Placement of components within an IMTA system is reliant upon not only the biotic capacity of organisms within the system, but also upon physical characteristics of the surrounding

environment. The physical environment surrounding an aquaculture system, IMTA stations in particular, is imperative to success of the farm. Hydrodynamic properties influence the supply of fresh water to a farm, as well as the dispersal of waste from a farm.

The dispersion of fecal pellets and feed particles is highly dependent on the local hydrodynamic regime, as well as the nature of the particles themselves. Heavy particles are subject to rapid sedimentation, whereas less dense or finer particles are likely to travel some distance from a fish farm, depending on the current. The distance from a farm which these particles are transported affects the level of impact that they may have on the surrounding environment and local marine communities (Kutti et al., 2007).

Influence of cage-arrays on current flow can result in highly complex, near-field hydrodynamics, and detailed knowledge of these effects are important considerations for numerous aquaculture management criteria. Current-flow in and around aquaculture cages affects volumetric loading of nutrient waste, attachment potential of ectoparastations, and the rate of dissolved oxygen supply (Page et al., 2005). Current flow and direction is of interest in the placement of co-culture species to optimize nutrient delivery in open-water Integrated Multi-Trophic Aquaculture (IMTA) systems.

Consideration in farm placement, and extractive organism arrangement, must be given to physical parameters unique to each specific site. Aquaculture engineering involves adaptations of farm installation designs and operation protocols for a variety of challenging physical factors, including currents and wave action, the ability to anchor structures, possible shipping routes, migration routes for marine mammals, and access to resources such as processing plants and resupply locations (North, 1987). Solutions to these challenges involve costs, which have implications for market scale and profits (NOAA, 2008).

By taking logistical concerns into account, as well as hydrodynamic properties of a site, the establishment of an IMTA system has the potential to harness nutrients within a system to create a more sustainable and economic operation as a whole. However, the specific abilities and requirements of extractive organisms must also be considered. In order to design an ecologically sound IMTA system, the capacity of organic extractive components to integrate farm waste into their diet must be defined and understood, so

that generalizations may be applied to the establishment of IMTA systems in a variety of unique conditions. A strong base of literature exists to support the link between filter feeding organisms, and consumption of fish farm waste, with a majority of research focusing on the relationship between Blue Mussels (*Mytilus edulis*) and Atlantic Salmon (*Salmo salar*).

Blue mussels (*Mytilus edulis*) grown adjacent to Atlantic salmon (*Salmo salar*) cages, such as those that are part of open-water Integrated Multi-Trophic Aquaculture (IMTA) systems 'clear' fish feed and fish fecal particulates (Neori et al., 2007). Reported increases in growth rates associated with these mussels compared to those grown at locations away from the influence of fish farms has been well documented (Lander, 2006). Absorption efficiency (AE) and percent digestion of the dietary organic component ingested by the mussels have also been studied in a laboratory setting (Reid, 2010).

However, in-situ quantification of the contribution of farm waste to the diet of filter feeding bivalves is necessary for determining the efficiencies of IMTA systems, the delivery of organic load to secondary niches (e.g. deposit feeders placed under shellfish rafts to consume bio-deposits) and to develop metrics for the ecological sustainability of IMTA. If, for example, bivalves are digesting little of the fish feces they ingest, the feces may be simply repackaged as biodeposits, with little reduction in the solid organic nutrient load from the system. Previous studies have determined that oysters can be successfully incorporated into integrated multi-trophic aquaculture systems with finfish (Cheshuk et al., 2003) based on growth of oysters to marketable size. However, whether fish farm waste contributes to the success of such integrated systems has not been fully quantified. It is therefore necessary to develop methods to determine if organisms, both in an integrated system and in the wild, are consuming fish farm waste.

This paper examines the ability of Pacific oysters (*Crassostrea gigas*) to incorporate fish farm waste into their diet in a variety of locations around a fish farm. By looking at the direct relationship between farm waste and consumption by organic extractive bivalves in an in-situ environment, over the course of one year, this paper aims to build upon a base of literature which has focused primarily on the use of growth data, lab studies, and modeling to establish the relationship between extractive organisms and

fish farm waste consumption. By providing a direct link between farm waste and oyster diets, a relationship can be established, and data can be used in the design of new IMTA systems.

By examining the ingestion percentage of filter feeding bivalves (*C. gigas*) through stable isotope analysis, a direct link can be established between filter feeding bivalves, and filtration of farm waste. Food sources of a filter-feeding bivalve species (*C. gigas*) under consideration for use as an organic extractive species within Integrated Multi-Trophic Aquaculture (IMTA) systems can be determined using stable isotope analysis. In order for extractive components to effectively uptake aquaculture waste, the relationship between the spatial and temporal patterns of filtration must be understood. Stable isotopes, specifically stable nitrogen and carbon species, have been identified as effective tools for measuring the flow of nutrients through biotic interactions. In this instance, stable isotope analysis was used in order to determine the variance in uptake of waste between oysters in and around a Sablefish (*Anopoploma fimbria*) farm, and those at a reference site.

Stable isotope analysis has been used to determine sources of nutrition for consumers, to study trophic relationships among organisms and to trace waste products from aquaculture (Yokoyama et al., 2002). The use of either fatty acids or stable isotopes as tracers relies on the food source and the consumer having distinct signatures. Stable isotope ratios from food sources are incorporated into animal tissues following assimilation (Pinnegar, 1999). Generally, there is enrichment of the heavier isotope forms (13C, 15N) as the lighter isotopes (12C, 14N) are preferentially utilized during metabolism (Dubois et al., 2007). Thus, the assimilation of the food can be measured by a shift in the stable isotope signature in the direction of the food source (Dalsgaard et al., 2003). Both stable carbon (δ13C) and nitrogen (δ15N) isotopes have been used to trace consumption. It is commonly accepted that the various tissues of an organism will respond to a change in diet at different rates depending on, for example, the turnover rate of the tissue (Tieszen et al., 1983).

Muscle tissue of *C. gigas* has been shown to respond to a switch in algal diet in approximately 20 days for both δ13C and δ15N (Dubois et al., 2007). Differences in tissue response times have been shown for other bivalve species as well (Paulet et al.,

2006). In order to take this lag time into account, and to develop a profile of *C. gigas* waste incorporation abilities on a seasonal basis, sampling was carried out every three months over the course of one year.

2.3 **Introduction-Temporal**

The marine aquaculture sector is growing rapidly, with an average annual growth rate of 6.9% (FAO, 2015). Aquaculture systems which combine fed aquaculture species (finfish), with inorganic extractive aquaculture species (seaweeds) and organic extractive species (suspension and deposit feeders) cultivated in proximity have been drawing increasing attention from researchers, industry and policy makers as a promising opportunity for large-scale expansion of the aquaculture industry. Such systems, described as integrated multi-trophic aquaculture (IMTA), could significantly increase the sustainability of aquaculture, based on a number of potential economic, societal and environmental benefits.

These benefits include the recycling of waste nutrients from higher trophic-level species into production of lower trophic level crops of commercial value. IMTA offers the potential for economically viable products to be cultivated in tandem, using the excess nutrients from one (finfish) to increase growth in another (extractive organic and inorganic species). Off the coast of British Columbia, in Kyuquot Sound, Sablefish (*Anapoploma fimbria*), Sugar Kelp (*Saccharina latissima*), and Qualicum Bay Scallops (*Patinopecten corinus*) are currently being raised in an IMTA system. In order to define the relationship between filter feeding bivalves and waste from finfish aquaculture, stable isotope analysis may be employed to quantify the importance of farm waste to the shellfish diet. Defining this relationship will help determine ideal placement of extractive organisms, as well as analyze their efficiency within an IMTA system on a seasonal basis. Pacific Oysters were used in this research for their feeding habits and ability to survive at varying depths within the water column. Pacific Oysters are an important aquaculture species in BC, and the ability to raise them in an IMTA system offers promising returns.

In 2013, Canada produced 11,190 ton of farmed oysters valued at CAD $23.6 million. Oysters accounted for 26% of the total shellfish production in Canada

(AquaStats, 2013). 64% of the volume and 43% of the value was accounted for by Pacific oysters produced in British Columbia. The remainder was accounted for by Atlantic and European oysters produced in Atlantic Canada (AquaStats, 2013). In 2014, 30% (3,304 tons) of the Canadian farmed oyster production was exported for a value of CAD $23.7 million; 89% were exported as live, fresh oysters. The United States is the primary export market for Canadian farmed oysters (87%; DFO, 2014). The demand is currently expanding rapidly and exceeds Canada's current supply. In an effort to meet this demand, many growers plan on major production expansions.

Oyster culture is one of the most prominent forms of marine aquaculture in the US as well. In 2011, 31 million pounds of oysters were harvested, with a dockside value of approximately $135 million (National Marine Fisheries Service, 2012). That may be considered a low estimate of the overall value of the oyster due to its failure to account for the ecological value of the fish species that use oyster reefs as a food source and nursery habitat, as well as the coastal protection and water-quality services oyster reefs provide. Factoring in both the economic and ecologic value of oyster reefs may increase that number into the billions. Pacific oysters are native to coastal Japan, but are an important aquaculture species on the West Coast of North America, and can be found from Southeast Alaska to Baja California. *C. gigas* is cultivated primarily on oyster farms in protected coastal estuaries; however, some wild beds exist in Washington and British Columbia. *C. gigas* prefers to live on hard surfaces in sheltered waters within the intertidal zone. However, they can be grown in a variety of conditions, and can survive in salinities between 10 and 32 parts per thousand, and temperatures of -1 to 35° Celcius (Helm, 2006).

In shellfish ecosystems on large intertidal mud flats, the food supply available to filter-feeding bivalves may consist largely of benthic diatoms. These organisms develop in the upper millimeters of sediments exposed to light, and form biofilms on the surface. They can become part of the phytoplankton once suspended by tidal currents and the interaction of wind and waves with the bottom (Ravail-Legrand et al., 1993). In some areas, microphytobenthos may represent up to 50% of the microalgae in the water column (Maclntyre et al., 1996). Gut content studies have shown that microphytobenthos are ingested by bivalves such as oysters and mussels (Newell et al., 1989). Analysis of the

carbon isotopic composition of oysters has also clearly but indirectly indicated that benthic microalgae can be a significant food source for oysters.

An important characteristic of bivalve feeding, when considering their incorporation within an IMTA system, is the presence of selective mechanisms at different steps of particle processing (Ward et al., 1998). The composition of suspended particulate matter in seawater can actually be modified by filter feeding bivalves through three main mechanisms: retention of particles on the gills, pre-ingestive selection on gills and/or labial palps, and differential absorption in the gut, (Ward et al., 1998). Retention on bivalve gills is at least partially dependent on particle size since the ctenidium does not retain the smallest particles with 100% efficiency (MacDonaldand Ward, 1994).

Past research suggests that oysters grown in the Mediterranean Sea do not retain the smallest cells, thereby favoring their development and altering the structure of the microalgal community. The ability to sort particles on their gills and/or labial palps before ingestion has been ascribed to many bivalve species, and the mechanisms involved have been thoroughly investigated (Ward et al., 1998).

Pre-ingestive selection can occur between inorganic and organic particles, and bivalves are also able to discriminate between different species of microalgae (Bougrier et al., 1997). Differential absorption has been described in different molluscan species as well. Nicotri (1977) showed that absorption efficiencies in herbivorous gastropods differed depending on the species of diatom consumed, and Shumway et al. (1985) found that the filter-feeding bivalve *Crassostrea virginica* practiced selective absorption among different species of microalgae. Selective filter feeders have the ability to affect the composition of the microalgal community, which suggests their ability to impact the distribution of particles suspended in the water column. This is of particular interest to IMTA design and management. The diet of Pacific oysters in an IMTA system may be modified by selective retention on bivalve gills, with pseudofeces and feces likely modified after pre-ingestive and post-ingestive selection, respectively. This paper aims to quantify the percent composition of aquaculture waste in the diet of *C. gigas*, and the relationship between aquaculture waste consumption and the abundance of particulate organic matter (POM) in the water column, on a seasonal basis.

On the west coast of British Columbia, there are large inputs of upwelled water (and nutrients) to the continental shelf from spring through autumn. Phytoplankton biomass is high, especially in mid to late summer, and is ample to support the growth of herbivorous zooplankton. Despite high food availability and relatively low predation pressure, surface layer zooplankton populations decline on the continental shelf from late spring through autumn, while offshore zooplankton populations increase slowly. A high washout rate due to upwelling and subsequent seaward and alongshore transport is the most plausible explanation. For the continental shelf in summer, the advective component of population turnover is probably larger than local predation mortality.

The continental shelf and slope waters off of Vancouver Island, British Columbia, are near the northern end of an extensive eastern boundary current/coastal upwelling domain that stretches south to Baja California. The entire system is characterized by strong alongshore and cross-shore advective throughput; on the large scale, this is dominated by the average equatorial flow of the California Current and at smaller scales by coastal upwelling circulation (Thomson and Ware, 1988). More locally, the oceanography of the southern BC continental margin has been studied regularly and intensively since the seventies.

Even in comparison with other rich mid-latitude shelf and eastern boundary current regions, the biological productivity of the southern BC shelf is very high (Thomson and Ware, 1988). Phytoplankton photosynthesis rates exceeding 20 mg Cm2 are very common throughout the spring, summer, and early autumn. This primary productivity is supported by input of inorganic nutrients via a number of physical processes including longshore transport from the arctic, local wind-driven upwelling, interaction of mesoscale currents with bottom topography, tidal mixing, and estuarine circulation in the Juan de Fuca Strait (Crawford and Dewey, 1989).

In 2012, the BC wild fishery industry produced 194,300 tons of fish and shellfish, consisting of 33,200 tons of salmon, 25,200 tons of herring, 117,300 tons of groundfish, and 18,600 tons of shellfish. The 2012 landed value for wild fish totaled $364 million, composed of $57 million of salmon, $47 million of herring, $153 million of groundfish, and $107 million of shellfish (DFO, 2013). These numbers suggest that a major fraction of the regional productivity is transferred to higher trophic levels. Many features of the

physical circulation are spatially localized and strongly associated with the coastline and bottom topography.

The continental shelf off southern Vancouver Island is wider and topographically more complex than neighboring coastal regions to the north and south. The southern and widest portion of the shelf is deep (>120m) and is cut by a submarine canyon system (>250 m) extending seaward from the Juan de Fuca Strait. The bottom shoals abruptly off the southeast corner of Barkley Sound forming a series of banks (70-100 m) and semi-enclosed basins (150- 200 m). Further up the coast, the shelf narrows gradually and the outer banks merge with coastal shallows. At the shelf break, upper layer alongshore currents are strong (often >40 cds to depths of about 50 m). The direction of flow is seasonal and is toward the southeast in summer and toward the northwest in winter (Thomas and Emery, 1986).

Changes in current direction accompany the spring and fall transitions in the large-scale weather pattern. Below about 150m, there is a slower and more persistent flow toward the northwest. Water properties suggest that this is a northward extension of the California Undercurrent (Mackas et al., 1987). Although on average both the Shelf Break Current and the California Undercurrent parallel the shelf break, dynamic instabilities and interactions with local bathymetry generate frequent meanders and eddies on the margin of the shelf (Hakeda et al., 1984). Especially in summer, upper layer water properties exhibit higher surface temperature, lower dissolved nutrients, and decreased phytoplankton biomass.

Within the core of the Shelf Break Current, summer levels of dissolved nutrients and phytoplankton biomass are often elevated due to the combined effects of shelf-edge upwelling and alongshore advection (Sirnard and Mackas, 1989). The two most obvious seasonal signals are summer warming of coastal waters, and significant cooling of the deeper layers due to the strong summertime intrusion of cold, high-salinity upwelled water. The surface layer warming is strongest in the outer shelf region and weakest in the southern shelf gyre region. The lowest surface salinity occurs in spring along the entire coast of BC. This timing is not attributable to the seasonality of coastal freshwater input, since it occurs during a seasonal minimum in total precipitation and runoff (Freeland et al, 1984).

Changes in salinity correlate better with the timing of the seasonal upwelling, and in turn with the seasonality of alongshore winds and currents. In mid- and high-latitude ecosystems, the annual seasonal cycle repeats every year with more or less the same amplitude and phase (Denman et al., 1989). The ability of organisms to deal with environmental fluctuations on a seasonal basis varies not only with the frequency and amplitude, but also with the predictability of the fluctuation. Most organisms have developed life history, migratory, and other evolutionarily adaptive strategies that minimize their risk during predictably unfavorable parts of the seasonal cycle. However, sessile organisms (such as oysters during the adult stage of their life cycle) have developed strategies to feed selectively, thus enabling them to exert some control over diet even in unfavorable conditions.

Oysters have well documented "growing seasons," as a result of feeding on abundant particulate organic matter, which is characteristic of the summer months (NOAA, 2005). Due to the abundance of particulate organic matter during certain months, and the selective feeding habits of oysters, it may be possible that their contributions within an IMTA system vary on a seasonal basis in response to environmental factors. This research aims to quantify the relationship between fish farm waste, and oyster diets, on a seasonal basis, using stable isotope analysis.

Stable isotopes, specifically stable Nitrogen and Carbon species, have been identified as effective tools for measuring the flow of nutrients through biotic interactions. In this instance, stable isotopes were used to determine the annual extractive contribution of oysters within an IMTA system as well as the variance in uptake of nutrient waste on a seasonal basis. These measurements were obtained through the analysis of δ15N and δ13C values within the extractive species *C. gigas*. Oysters were deployed in lantern nets within the nutrient plume of farmed *A. fimbria* within an IMTA system. A reference site was also established 100m from the fed component, where past work has shown no trace of aquaculture waste. Food sources analyzed include aquaculture system waste in the form of fecal matter and feed pellets, while natural sources include suspended particulate organic matter.

2.4 Methods:

2.4.1 Site description

Data collection for this research was conducted at the Kyuquot SEAfoods Inc. IMTA site in the northwest region of Vancouver Island, British Columbia (50°3‘10‖N 127°18‘45‖W) just off Surprise Island, Kyuquot Sound. It lies approximately 4.5 kilometers from the village of Kyuquot. The studied IMTA system is located in the deepest part (25m) of a shallow protected embayment between Vancouver Island and Surprise Island. The small channel to the southwest of the embayment connects to the larger Crowthier channel, which opens to the Pacific Ocean.

To the east the embayment opens into Kyuquot Sound, which is a deep coastal fjord. During flood tide, the water generally flows in from the southwestern channel and out towards Kyuquot Sound. The opposite occurs during ebb tide. The unique bathymetry of the embayment creates a gyre, which reduces current velocities, causes unidirectional flow and recirculates water in the western sector of the embayment. The exposed coastline to the west of the IMTA site is one of the most intense wind and wave environments in the province of British Columbia. While the site is sheltered from waves, it is exposed to strong southeasterly winds during the winter months (Coast and Marine Planning Branch, 2003).

Additionally, the area is characterized by high rainfall with the annual mean total precipitation being greater than 4000 mm. The majority of this precipitation occurs in late fall and winter with total precipitation for January usually being greater than 400 mm (Natural Resources Canada, 2009). This precipitation results in runoff, which can lead to the development of a highly stratified water column with fresh water surface layers laden with suspended materials (Coast and Marine Planning Branch, 2003). However, during strong wind and tidal events considerable mixing can occur, de-stratifying the water column. In general, stratification develops during the calm summer months due to thermal heating of the surface waters. In spring and summer, phytoplankton production within the coastal inlets on the west coast of the island can be limited due to low water exchange, stratification and reduced nutrient availability (Hay et al., 2003). Yet, during these seasons, offshore diatom blooms (*Skeletonema costatum*) are commonly carried

into the inlets via wind forcing. Similarly, Emiliania huxleyi (*E. huxleyi*), an opportunistic coccolithophore species, can also be carried into these inlets where they can form isolated blooms over periods of high thermal stratification (Taylor & Haigh, 1996).

The system spans across a 5 km^2 marine study area, which is characterized by circular tidal flow. At approximately 30 m, it lies primarily in a photic zone. British Creek, a stream in close proximity to the site, discharges relatively low flows of fresh water into the system. Current flow rate is low, and runs laterally through a series of seven sablefish cages (50 x 50 ft2, 60 ft deep) ranging approximately 4,500 to 10,000 fish per cage. However, the number of fish varies by season and site supervision. The current continues through to a series of shellfish droplines spaced one metre apart across a raft system that is approximately 14m across and 75m long. Each dropline has 12 tiers, the top of which is approximately five metres deep. Each tier houses approximately 25 to 50 Pacific scallops (*Patinopectin yessoensis).* The current then passes through a number of sugar kelp (*Saccharina latissima*) lines. The seafloor is dominated by a muddy substrate. An additional site within Kyuquot Sound, located approximately 500 m northeast of the IMTA research and development site, was used as a sampling reference site.

2.4.2 Experimental Design

In order to examine the importance of farm-derived waste to the diet of extractive organic components within an IMTA system, Pacific oysters (*Crassostrea gigas*) were arranged in lantern nets, suspended at varying depths and distances from the fed component. Oysters were selected as a target organism for this study due to their value as a commodity within the aquaculture industry, suitability to site conditions, and for their selective feeding habits (Sarkis, 2007). Lantern nets were hung at a depth of 6m at stations located at horizontal distances of 0m, 15m, and 30m adjacent to the fed component, directly in the current exiting the fish nets. A fourth site was set up as a reference, out of the influence of aquaculture-derived waste. These stations were designated as station one (0m), station two (15m) station three (30m) and station four (reference). Nets were also suspended at 18m depth at each station within the IMTA

system, as well as the reference site. Hanging lines consisted of three lantern nets, each containing 10 juvenile Pacific oysters (*Crassostrea gigas*) from Fanny Bay, BC.

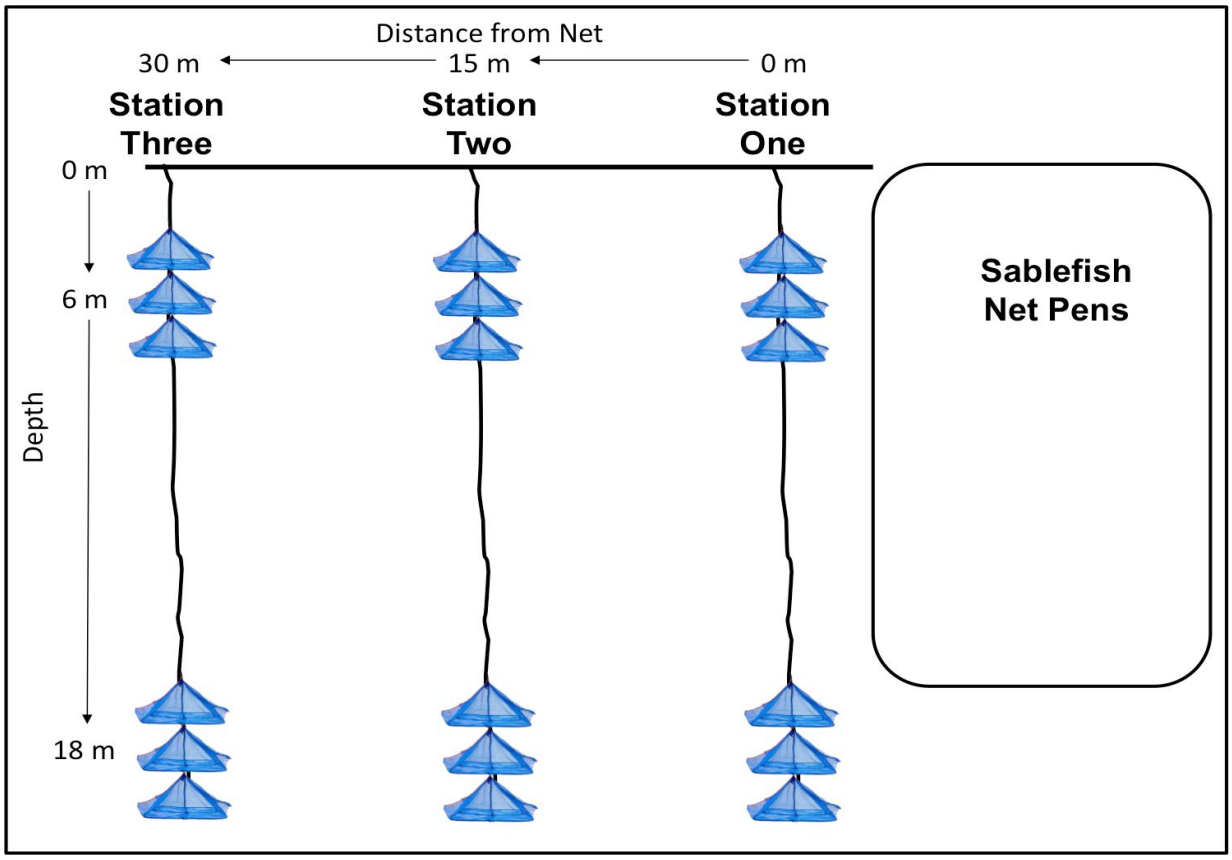

Figure 2.1 Organization of research stations within an IMTA site.

2.4.3 Collecting and processing

At the IMTA site, five individual Pacific oysters (*Crassostrea gigas*) were collected from each depth (6m, 18m) and distance from the fish nets (stations one, two, and three at distances of 1m, 15m, 30m respectively), every third month over the course of one year (Dec. 2013-Dec. 2014). Five individuals were also collected from each depth at the reference site (station four). All samples were dissected and processed in an on-site laboratory. Prior to dissection, samples were rinsed with distilled water. Only tissues with low variability were sampled, which include muscle (Perga & Gerdeaux, 2005; Lajtha & Michener, 2007) of the sablefish and adductor muscle (dissected from the shell and soft body) of the oysters using a scalpel or surgical blade (Dubois et al., 2007). Sampling

tissues with low variability ensures that isotopic values can be biased towards feeding patterns of the recent past, over the course of a few months. Many studies have demonstrated that different tissues reflect diet at different rates (Sarakinos et al., 2002). Muscle tissue samples were labeled and frozen for transport to the University of Victoria for processing.

Discrete water samples (2l) were pumped from a depth of 18m at a reference site, in order to analyze isotopic signatures of naturally occurring POM. Five replicates were prefiltered in situ through a 200-lm mesh sieve to remove large particles. The remaining water was refiltered through precombusted (450C for 4h) Whatman GF/F glass fiber filters (nominal pore size = 0.7 lm) to obtain fine POM (FPOM,<20 lm). Filters were stored in petri dishes and frozen for transport to the University of Victoria for processing. Once on site, samples were freeze dried and prepared for stable isotope analysis.

Fish feeds and feces were collected by using a sediment trap (50cm acrylic tube) deployed beneath the fish cages. These sediments were collected using a gravity corer of 5 cm diameter, and surface sediments (top 0.5 cm) were sliced for collecting sedimentary organic matter (SOM). All samples of muscle tissue, POM, and SOM were freeze-dried and homogenized by pulverizing with a ball mill (Retsch MM200 Mixer Mill, Hyland Scientific, WA), and then kept in a deep freezer (70C) until the isotope analysis. 1mg samples of all powdered tissues were weighed in tin containers (6 x 4 mm) in preparation for sampling via continuous flow isotope ratio spectrometry (CF-IRMS) analysis. Three replicates of each tissue sample were made for analysis in the mass spectrometer. Freeze-dried tissue not needed for analysis was catalogued and stored.

The powdered samples sealed in tin capsules and filter samples wrapped with tin disks were combusted at high temperature (1030 C) in a CHN elemental analyzer (vario MICRO cube, Hanau, Germany) and the resultant gasses (CO2 and N2) were introduced into a continuous-flow isotope ratio mass spectrometer (CF-IRMS; IsoPrime 100, Cheadle, UK) to determine their isotope ratios. The stable isotope data are expressed as the relative differences between the samples and conventional standard reference materials (Vienna Pee Dee Belemnite for carbon and atmospheric N2 for nitrogen), as follows:

$$(‰) = ([Rsample/Rstandard] -1) \times 10^3$$

where X is 13C or 15N and R is 13C/12C or 15N/14N. International standards of sucrose (ANU C12H22O11; NIST), Gaithersburg, MD) and ammonium sulfate ([NH4]2SO4; NIST) were used to calibrate the isotopic values as the reference material. The analytical precision for 20 replicates of urea was within 0.1‰ and 0.2‰ for d13C and d15N, respectively.

2.4.4 Isotopic Analysis

Isotopic measurements were performed at the University of Victoria. CF-IRMS analysis was completed using a Europa Hydra 20/20 continuous-flow isotope ratio mass spectrometer. Analytical error of δ13C and δ15N were determined using replicates of laboratory standards. Data results are expressed in the standard delta notation (parts per thousand (‰) deviations from standards including Pee Dee Belemnite (PDB) for δ13C and atmospheric N2 for δ15N) as:

$$\delta X = [(Rsample/Rstandard) - 1] \times 1000$$

where X is either carbon (C) or nitrogen (N) and R is the ratio of heavy to light stable isotope (13C/12C or 15N/14N). Triplicate samples were analyzed haphazardly and resulted in an analytical reproducibility of 0.97 ‰ for δ13C and 0.96 ‰ for δ15N.

When comparing isotopic signatures of consumers versus food sources over variable time scales, it is important to consider time-lags as isotopic compositions measured within the tissues of consumers do not necessarily reflect the diets of their food sources within a single time scale (Hobson & Clark, 1992). Consumers tend to exhibit less temporal variability in isotopic signatures than primary producers. Most temporal variability has been measured in consumer organisms with high tissue turnover rates, such as invertebrates and fishes with shorter life spans (Vizzini & Mazzola, 2003).

2.4.5 Statistics

An isotopic baseline of particulate organic matter in Kyuquot sound was established during the period of study, as well as aquaculture derived waste from the IMTA site, providing a reference point for results. Tissue samples were analyzed for δ13C and δ15N in order to determine the influence of IMTA derived waste on the diet of extractive

organisms at each station. In order to perform statistical analysis, all isotopic signature data were tested for normality using Kolmogorov-Smirnov test, and homogeneity of variance using Levene's test. A one-way ANOVA was performed to quantitatively estimate spatial variations between stations, and temporal variations between sampling dates. Tukey's post hoc test was used to test the significant differences among the d13C and d15N values of fish feeds, primary producers, and organic matter sources, as well as the differences between stations within the IMTA system. All statistical analyses were performed using SPSS software (version 12.0, Chicago, USA). Data are presented as mean ±SE.

2.4 Results

2.4.1 Isotope signatures of feed and organic matter sources

Feces of cage-cultured fish had isotope signatures that were distinguishable (more enriched δ13C but more depleted δ15N) from those of the fish feeds (-19.46± 0.09‰ vs. -21.15 ± 0.06‰; 8.41 ± 0.08‰ vs. 13.13 ± 0.05‰, respectively). Such an isotopic discrepancy between feed and feces has also been reported, reflecting differences in metabolic activity of fish species and composition of their feed (McGhie et al., 2000; Franco-Nava et al., 2004). Yokoyama et al. (2006) reported that cultured fish, which fed on artificial feeds, excrete feces containing specific undigested materials. Many studies also report no discrepancy between feed and feces (Mazzola & Sarà 2001; Ye *et al.* 1991; Sutherland *et al*. 2001), which suggests that this relationship is dependent upon the type of feed and species of fish. Despite the considerable δ15N depletion in feces of the cultured Sablefish compared with that of the fish feeds, both the feces' δ13C and δ15N signatures were significantly different from those of naturally occurring POM (-21.15 ± .06‰ vs -23.83 ± .08‰; 8.41 ± .08‰ vs 7.29 ± .09‰ respectively).

2.4.2 Isotopic signatures of C. gigas tissue samples by station

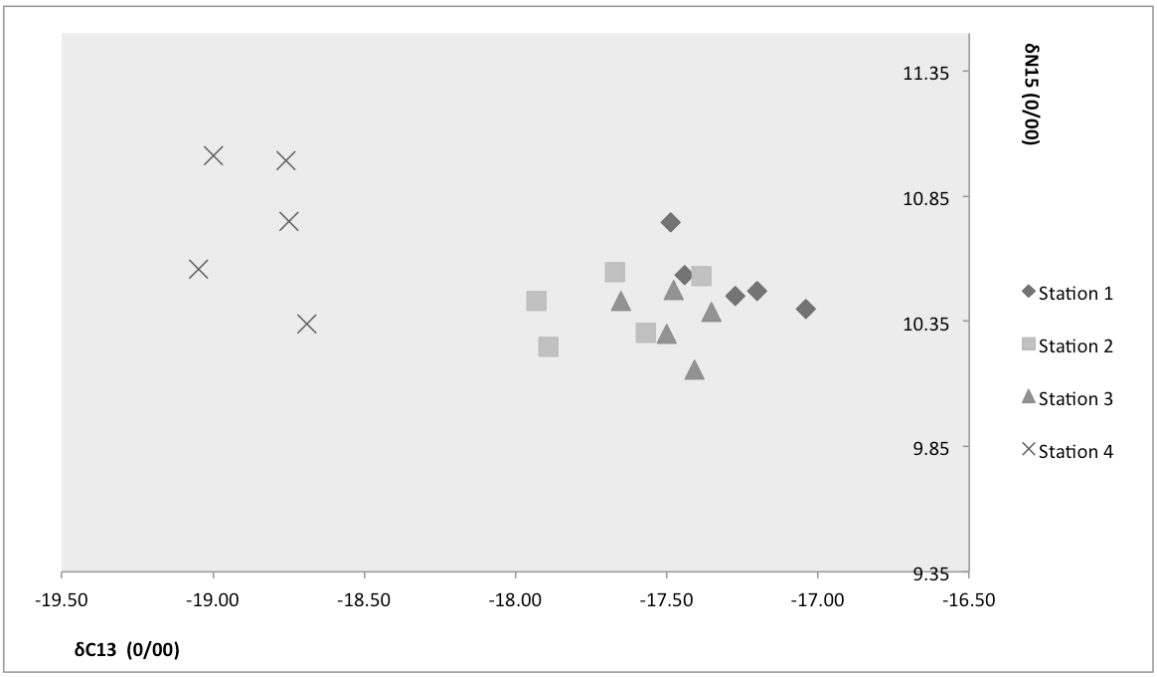

Figure 2.2. Dual isotope plot of δ13C and δ15N values for *C. gigas* tissue samples from four stations (three IMTA, one reference) at a depth of 6m, for the month of April.

The April δ13C values of *C. gigas* muscle tissue at a depth of 6m were: station one, -17.29 ± 0.08‰; station two, -17.69 ± 0.10‰; station three, -17.48 ± 0.05‰; station four (reference) -18.85 ± 0.07‰. The April δN15 values of *C. gigas* muscle tissue at 6m were: station one, 10.52 ± 0.06‰; station two, 10.41 ± 0.06‰; station three, 10.35 ± 0.06‰; station four (reference) 10.73 ± 0.13‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was used to test the significant differences among the δ13C values of each station. One way ANOVA ($F_{3,16}$=78.96, p=<.05). Station one vs station two (p=.012, p<.05), station one vs station three (p=.36, p>.05), station one vs station four (p=.001, p<.01), station two vs station three (p=.28, p>.05), station two vs station four (p=.001, p<.01), station three vs station four (p=.001, p<.01).

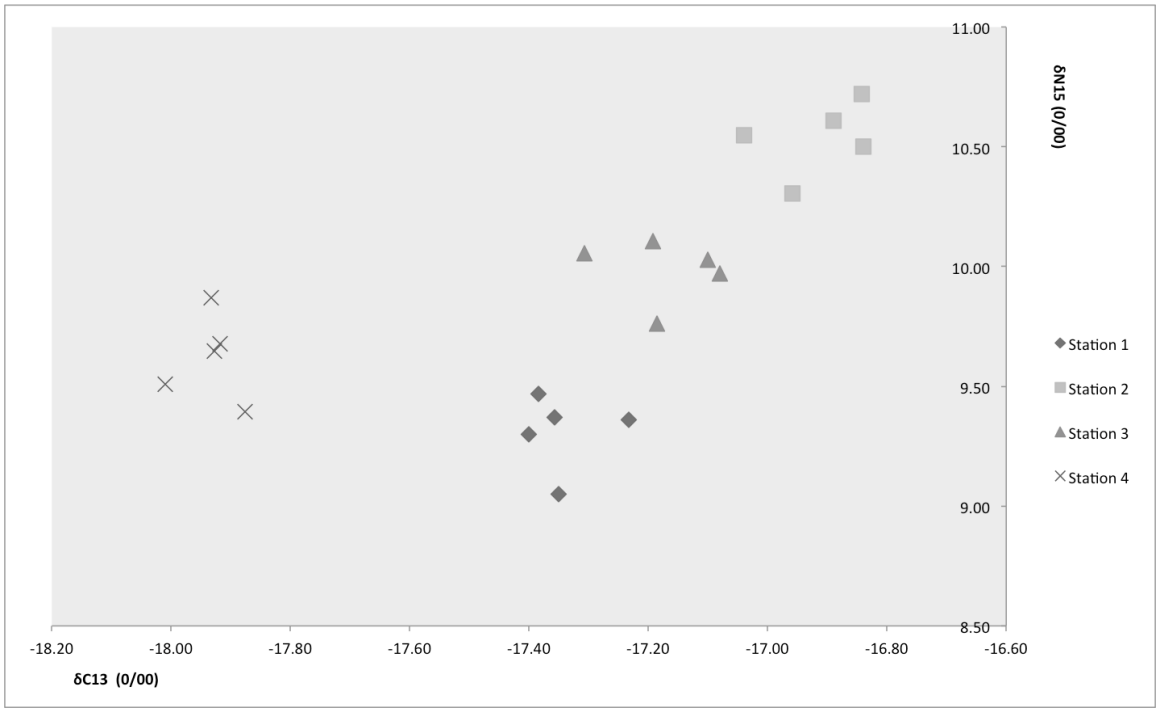

Figure 2.3. Dual isotope plot of δ13C and δ15N values for *C. gigas* tissue samples from four stations (three IMTA, one reference) at a depth of 18m depth, for the month of April.

The April δ13C values of *C. gigas* muscle tissue at a depth of 18m were: station one, -17.35± 0.01‰; station two, -16.91± 0.04‰; station three, -17.17± 0.04‰; station four (reference) -17.93 ± 0.02‰. The April δN15 values of *C. gigas* muscle tissue at a depth of 18m were: station one, 10.54 ± 0.02‰; station two, 9.23 ± 0.10‰; station three, 9.98 ± 0.06‰; station four (reference) 9.62 ± 0.08‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was used to test the significant differences among the δ13C values of each station. One way ANOVA ($F_{3,16}$=167.449, p=<.01) Station one vs station two (p=.001, p<.01), station one vs station three (p=.01, p<.05), station one vs station four (p=.001, p<.01), station two vs station three (p=.001, p<.01), station two vs station four (p=.001, p<.01), station three vs station four (p=.001, p<.01).

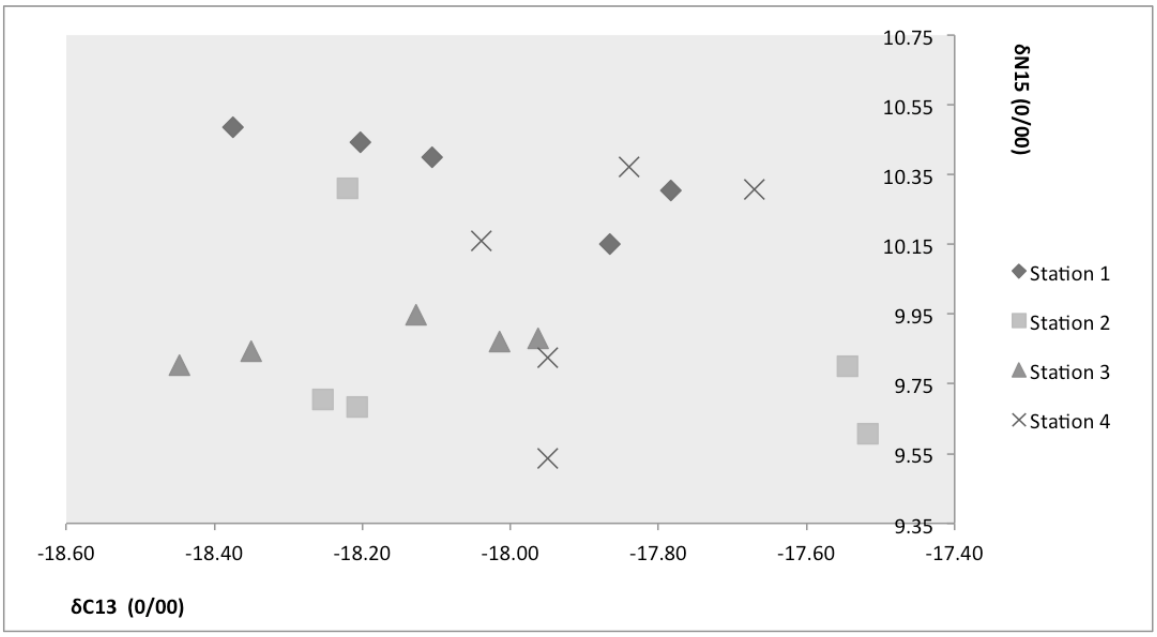

Figure 2.4. Dual isotope plot of δ13C and δ15N values for *C. gigas* tissue samples from four stations (three IMTA, one reference) at a depth of 6m, for the month of June.

The δ13C values of *C. gigas* muscle tissue at a depth of 6m in June were: station one, -18.01 ± 0.06‰; station two, -17.72 ± 0.03‰; station three, -18.03 ± 0.09‰; station four (reference), -18.24 ± 0.03‰. The δN15 values of *C. gigas* muscle tissue at a depth of 6m in June were: station one, 10.29 ± 0.04‰; station two, 10.14 ± 0.09‰; station three, 10.14 ± 0.09‰; station four (reference) 10.28 ± 0.07‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was used to test the significant differences among the δ13C values of each station. One way ANOVA ($F_{3,16}$=1.23, p=<.05). Station one vs station two (p=.88, p>.05), station one vs station three (p=.90, p>.05), station one vs station four (p=.69, p>.05), station two vs station three (p=.51, p>.05), station two vs station four (p=.90, p>.05), station three vs station four (p=.32, p>.05).

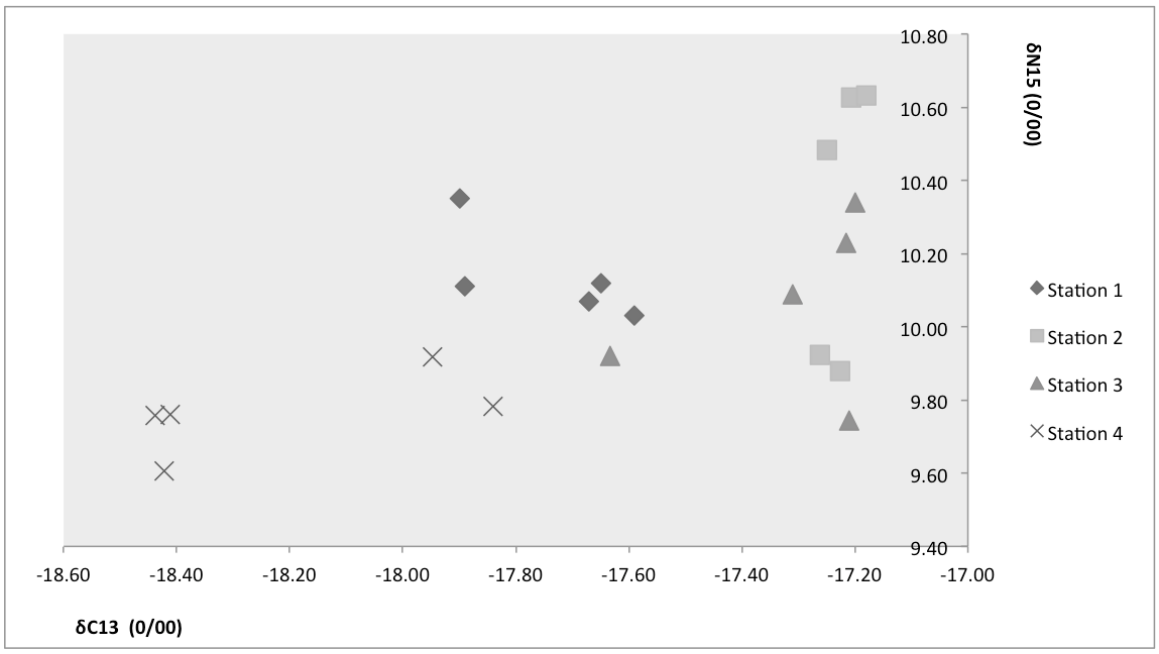

Figure 2.5. Dual isotope plot of δ13C and δ15N values for *C. gigas* tissue samples from four stations (three IMTA, one reference) at a depth of 18m, for the month of June.

The δ13C values of *C. gigas* muscle tissue at a depth of 18m in June were: station one, -17.61± 0.04‰; station two, -17.25± 0.04‰; station three, -17.47± 0.05‰; station four (reference), -18.3 ± 0.10‰. The δN15 values of *C. gigas* muscle tissue at a depth of 18m in June were: station one, 10.34 ± 0.07‰; station two, 10.20 ± 0.10‰; station three, 10.12 ± 0.07‰; station four (reference) 9.99 ± 0.06‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was used to test the significant differences among the δ13C values of each station. One way ANOVA ($F_{3,16}$=1.2, p>.05). Station one vs station two (p=1.01, p>.05), station one vs station three (p=.982, p>.05), station one vs station four (p=1.52, p>.05), station two vs station three (p=1.10, p>.05), station two vs station four (p=.51, p>.05), station three vs station four (p=2.51, p>.05).

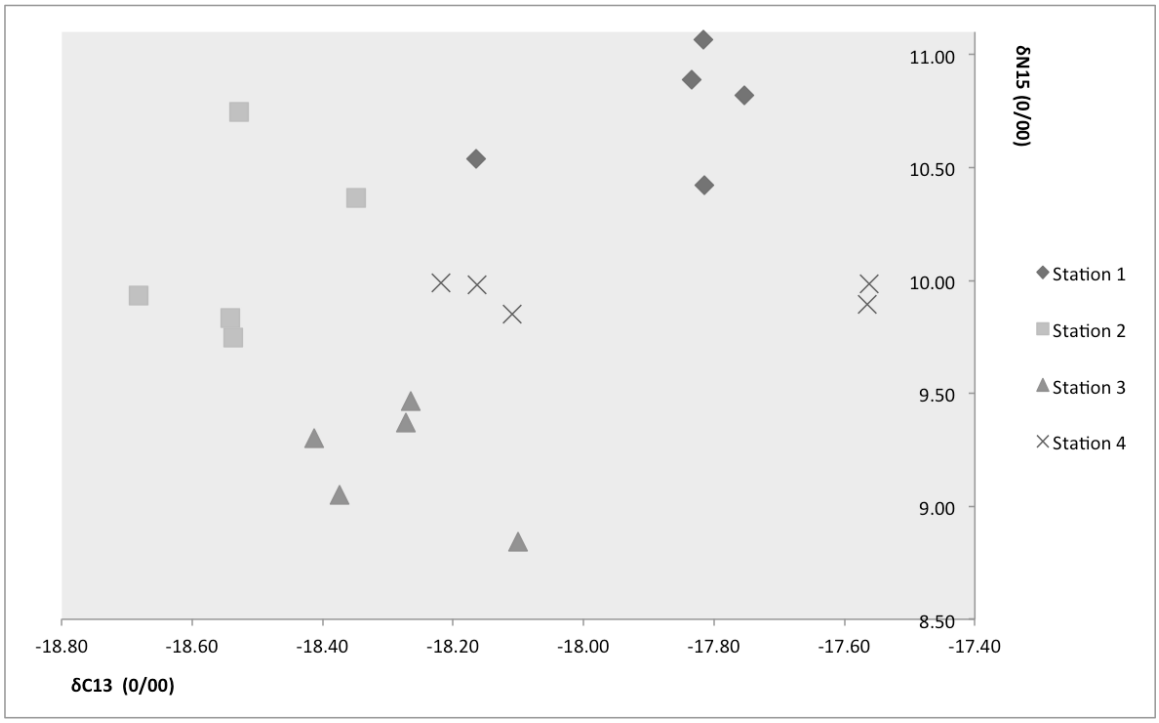

Figure 2.6. Dual isotope plot of δ13C and δ15N values for *C. gigas* tissue samples from four stations (three IMTA, one reference) at a depth of 6m, for the month of September.

The δ13C values of *C. gigas* muscle tissue at a depth of 6m in September were: station one, -17.96 ± 0.09‰; station two, -18.36 ± 0.06‰; station three, -18.15 ± 0.05‰; station four (reference) -18.24 ± 0.05‰. The δN15 values of *C. gigas* muscle tissue at a depth of 6m in September were: station one, 10.62 ± 0.06‰; station two, 10.84 ± 0.09‰; station three, 9.04 ± 0.07‰; station four (reference) 10.84 ± 0.11‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was used to test the significant differences among the δ13C values of each station. One way ANOVA ($F_{3,16}$=11.74, p=<.05). Station one vs station two (p=.0001, p<.01), station one vs station three (p=.03, p<.05), station one vs station four (p=.91, p>.05), station two vs station three (p=.26, p>.05), station two vs station four (p=.001, p<.01), station three vs station four (p=.06, p>.05).

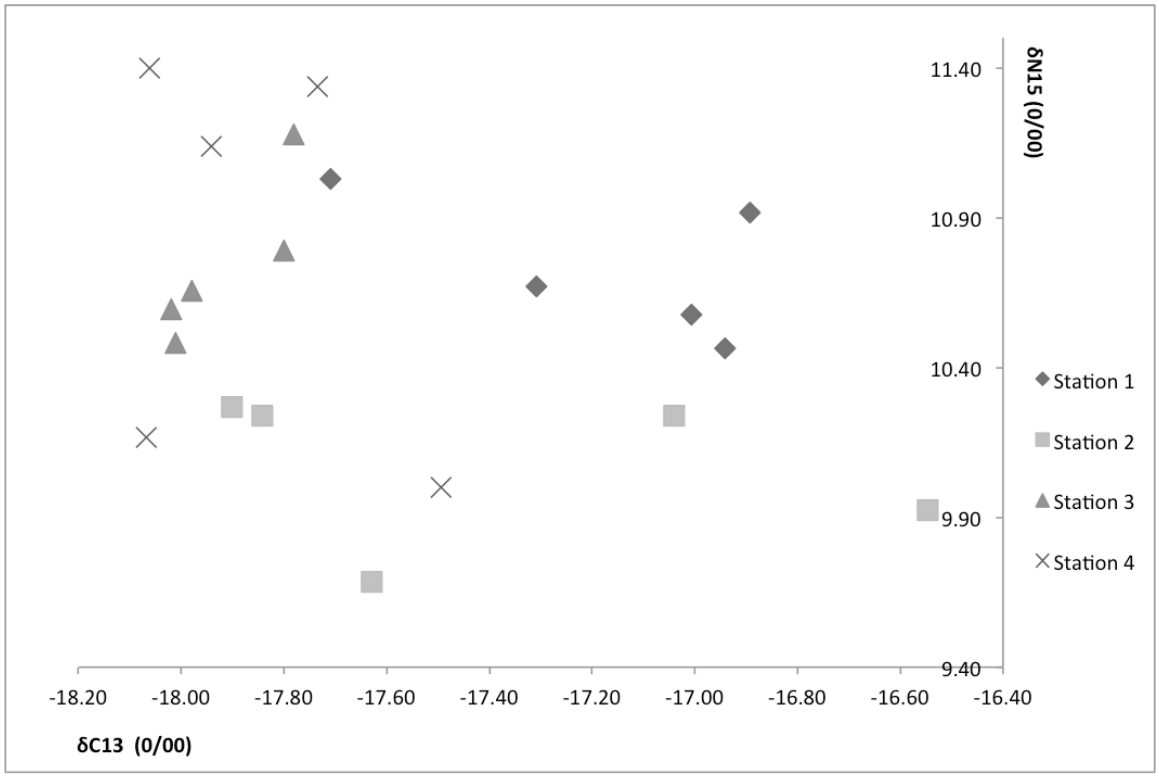

Figure 2.7. Dual isotope plot of δ13C and δ15N values for *C. gigas* tissue samples from four stations (three IMTA, one reference) at a depth of 18m, for the month of September.

The δ13C values of *C. gigas* muscle tissue at a depth of 18m in September were: station one, -17.59± 0.08‰; station two, -17.39± 0.11‰; station three, -17.90 ± 0.02‰; station four (reference) -17.78 ± 0.05‰. The δN15 values of *C. gigas* muscle tissue at a depth of 18m September were: station one, 10.73 ± 0.06‰; station two, 10.00 ± 0.12‰; station three, 10.84 ± 0.04‰; station four (reference) 10.60 ± 0.13‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was used to test the significant differences among the δ13C values of each station. One way ANOVA ($F_{3,16}$=4.96, p=<.02). Station one vs station two (p=.76, p>.05), station one vs station three (p=.02, p<.05), station one vs station four (p=.04, p<.05), station two vs station three (p=.14, p>.05), station two vs station four (p=.22, p>.05), station three vs station four (p=.91, p>.05).

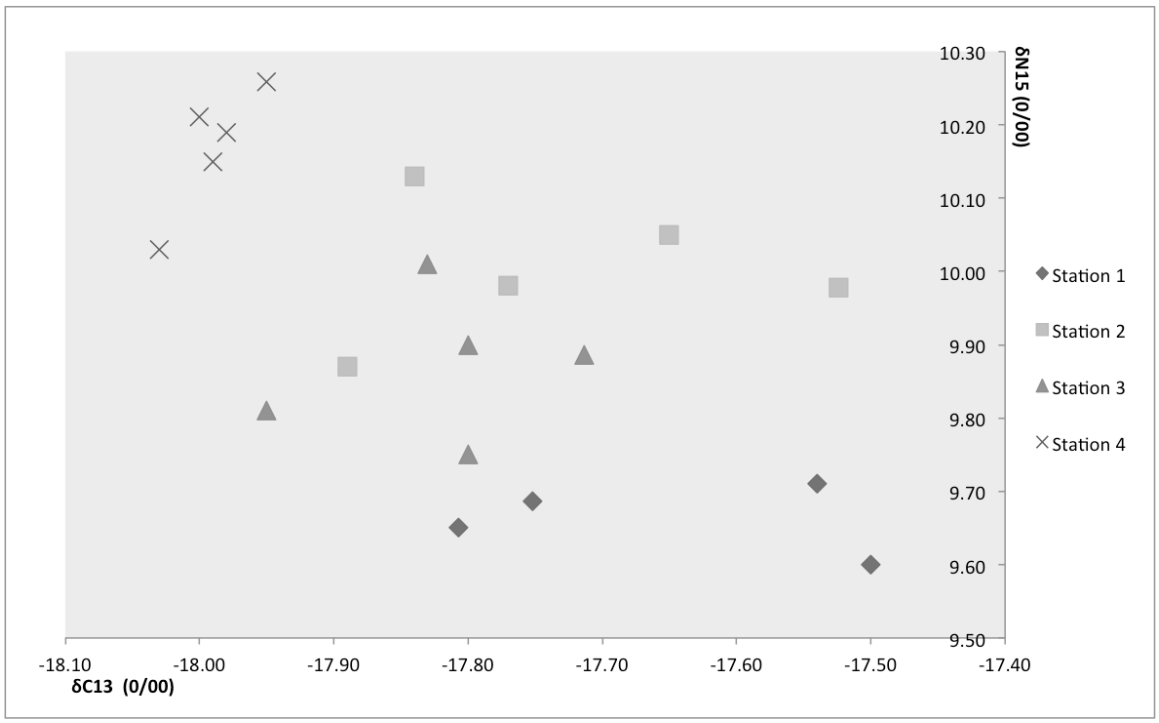

Figure 2.8. Dual isotope plot of δ13C and δ15N values for *C. gigas* tissue samples from four stations (three IMTA, one reference) at a depth of 6m, for the month of December.

The δ13C values of *C. gigas* muscle tissue at a depth of 6m in December were: station one, -17.66± 0.06‰; station two, -17.75 ± 0.07‰; station three, -17.82 ± 0.04‰; station four (reference) -17.99 ± 0.01‰. The δN15 values of *C. gigas* muscle tissue at a depth of 6m in December were: station one, 9.62 ± 0.05‰; station two, 10.02 ± 0.06‰; station three, 9.87 ± 0.04‰; station four (reference) 10.02 ± 0.04‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was used to test the significant differences among the δ13C values of each station. One way ANOVA ($F_{3,16}$=8.25, p=<.01). Station one vs station two (p=.71, p>.05), station one vs station three (p=.15, p>.05), station one vs station four (p=.001, p<.01), station two vs station three (p=.62, p>.05), station two vs station four (p=.02, p<.05), station three vs station four (p=.10, p>.05).

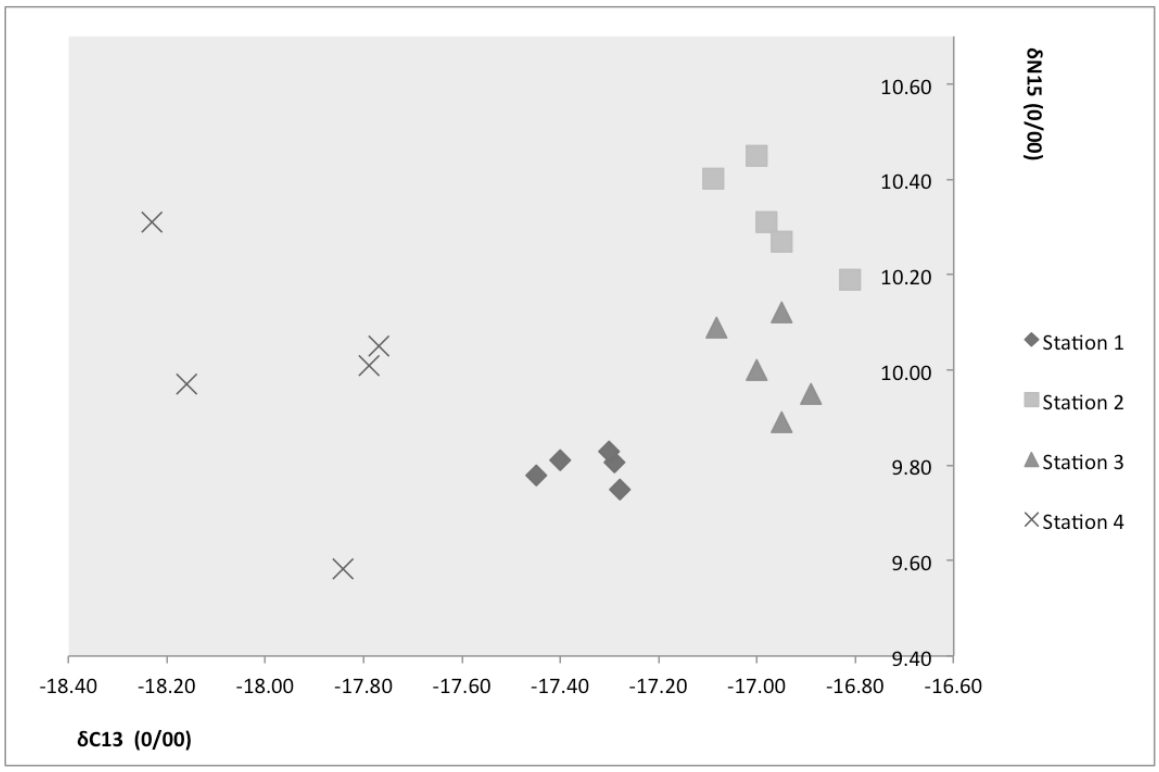

Figure 2.9. Dual isotope plot of δ13C and δ15N values for *C. gigas* tissue samples from four stations (three IMTA, one reference) at a depth of 18m, for the month of December.

The December δ13C values of *C. gigas* muscle tissue at a depth of 18m were: station one, -17.34 ± 0.03‰; station two, -16.99± 0.06‰; station three, -16.97 ± 0.03‰; station four (reference) -17.90 ± 0.12‰. The δN15 values of *C. gigas* muscle tissue in December, at a depth of 18m, were: station one, 9.8 ± 0.01‰; station two, 10.22 ± 0.08‰; station three, 10.01 ± 0.04‰; station four (reference) 9.98 ± 0.11‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was used to test the significant differences among the δ13C values of each station. One way ANOVA ($F_{3,16}$=62.79, p=<.05). Station one vs station two (p=.002, p<.01), station one vs station three (p=.002, p<.01), station one vs station four (p=.001, p<.01), station two vs station three (p=.91, p>.05), station two vs station four (p=.001, p<.05), station three vs station four (p=.001, p<.05).

April	δC13			δN15		
Sample Group	**Obs (*n*)**	**Mean**	**SE**	**Obs (*n*)**	**Mean**	**SE**
Shallow (6m)						
C. gigas (Station One)	5	-17.29	0.08	5	10.52	0.06
C. gigas (Station Two)	5	-17.69	0.1	5	10.41	0.06
C. gigas (Station Three)	5	-17.48	0.05	5	10.35	0.06
C. gigas (Reference)	5	-18.85	0.07	5	10.73	0.13
Deep (18m)						
C. gigas (Station One)	5	-17.35	0.01	5	10.54	0.02
C. gigas (Station Two)	5	-16.91	0.04	5	9.23	0.1
C. gigas (Station Three)	5	-17.17	0.04	5	9.98	0.06
C. gigas (Reference)	5	-17.93	0.02	5	9.62	0.08
Fish Feed	5	-19.46	0.09	5	13.13	0.05
Feces	5	-21.15	0.06	5	8.41	0.08
POM	5	-23.83	0.08	5	7.29	0.09

Table 2.1. δ13C and δ15N values for *C. gigas* tissue samples, as well as feed sources, from four stations (three IMTA, one reference) at depths of 6m and 18m, for the month of April.

June	δC13			δN15		
Sample Group	**Obs (*n*)**	**Mean**	**SE**	**Obs (*n*)**	**Mean**	**SE**
Shallow (6m)						
C. gigas (Station One)	5	-18.01	0.06	5	10.29	0.04
C. gigas (Station Two)	5	-17.72	0.03	5	10.14	0.09
C. gigas (Station Three)	5	-18.03	0.09	5	10.14	0.09
C. gigas (Reference)	5	-18.24	0.03	5	10.28	0.07
Deep (18m)						
C. gigas (Station One)	5	-17.61	0.04	5	10.34	0.07
C. gigas (Station Two)	5	-17.25	0.04	5	10.2	0.1
C. gigas (Station Three)	5	-17.47	0.05	5	10.12	0.07
C. gigas (Reference)	5	-18.3	0.1	5	9.99	0.06
Fish Feed	5	-19.46	0.09	5	13.13	0.05
Feces	5	-21.15	0.06	5	8.41	0.08
POM	5	-23.83	0.08	5	7.29	0.09

Table 2.2. δ13C and δ15N values for *C. gigas* tissue samples, as well as feed sources, from four stations (three IMTA, one reference) at depths of 6m and 18m, for the month of June.

September	δC13			δN15		
Sample Group	**Obs (*n*)**	**Mean**	**SE**	**Obs (*n*)**	**Mean**	**SE**
Shallow (6m)						
C. gigas (Station One)	5	-17.96	0.09	5	10.62	0.06
C. gigas (Station Two)	5	-18.36	0.06	5	10.84	0.09
C. gigas (Station Three)	5	-18.15	0.05	5	9.04	0.07
C. gigas (Reference)	5	-18.24	0.05	5	10.84	0.11
Deep (18m)						
C. gigas (Station One)	5	-17.59	0.08	5	10.73	0.06
C. gigas (Station Two)	5	-17.39	0.11	5	10	0.12
C. gigas (Station Three)	5	-17.9	0.02	5	10.84	0.04
C. gigas (Reference)	5	-17.78	0.05	5	10.6	0.13
Fish Feed	5	-19.46	0.09	5	13.13	0.05
Feces	5	-21.15	0.06	5	8.41	0.08
POM	5	-23.83	0.08	5	7.29	0.09

Table 2.3. δ13C and δ15N values for *C. gigas* tissue samples, as well as feed sources, from four stations (three IMTA, one reference) at depths of 6m and 18m, for the month of September.

December	δC13			δN15		
Sample Group	**Obs (*n*)**	**Mean**	**SE**	**Obs (*n*)**	**Mean**	**SE**
Shallow (6m)						
C. gigas (Station One)	5	-17.66	0.06	5	9.62	0.05
C. gigas (Station Two)	5	-17.75	0.07	5	10.02	0.06
C. gigas (Station Three)	5	-17.82	0.04	5	9.87	0.04
C. gigas (Reference)	5	-17.99	0.01	5	10.02	0.04
Deep (18m)						
C. gigas (Station One)	5	-17.34	0.03	5	9.8	0.01
C. gigas (Station Two)	5	-16.99	0.06	5	10.22	0.08
C. gigas (Station Three)	5	-16.97	0.03	5	10.01	0.04
C. gigas (Reference)	5	-17.9	0.12	5	9.98	0.11
Fish Feed	5	-19.46	0.09	5	13.13	0.05
Feces	5	-21.15	0.06	5	8.41	0.08
POM	5	-23.83	0.08	5	7.29	0.09

Table 2.4. δ13C and δ15N values for *C. gigas* tissue samples, as well as feed sources, from four stations (three IMTA, one reference) at depths of 6m and 18m, for the month of December.

2.4.3 Isotopic signatures of C. gigas tissue samples on a temporal scale

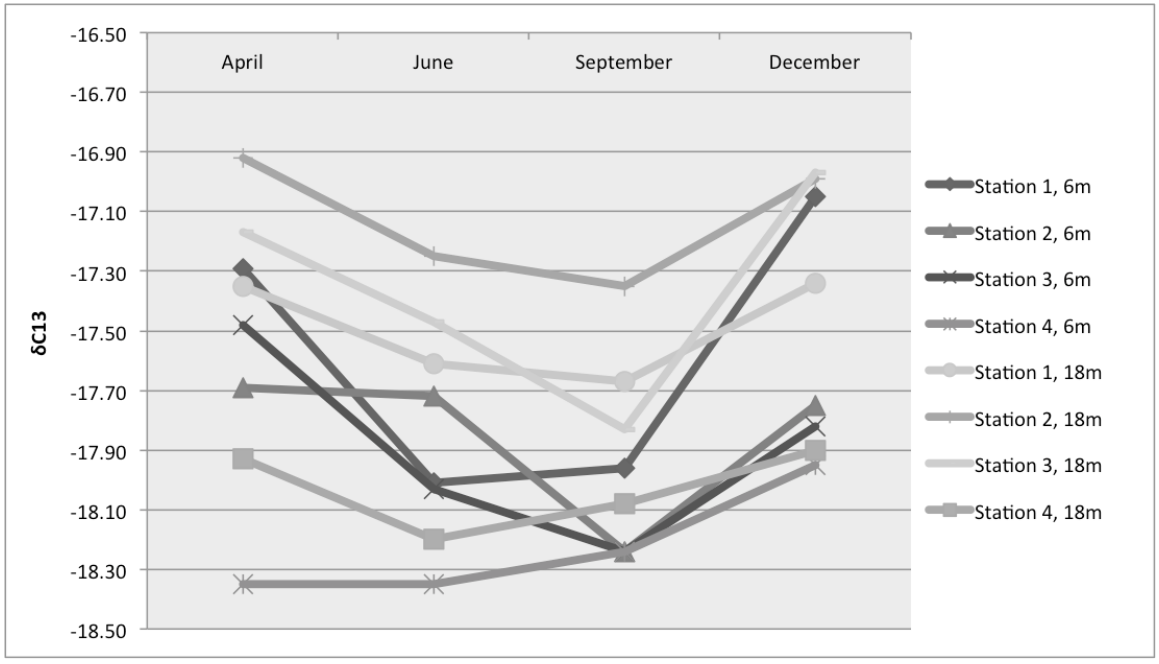

Figure 2.10. Mean δ13C values of *C. gigas* tissue samples from depths of 6m and 18m, during the sampling period (April-Dec, 2015), across all stations.

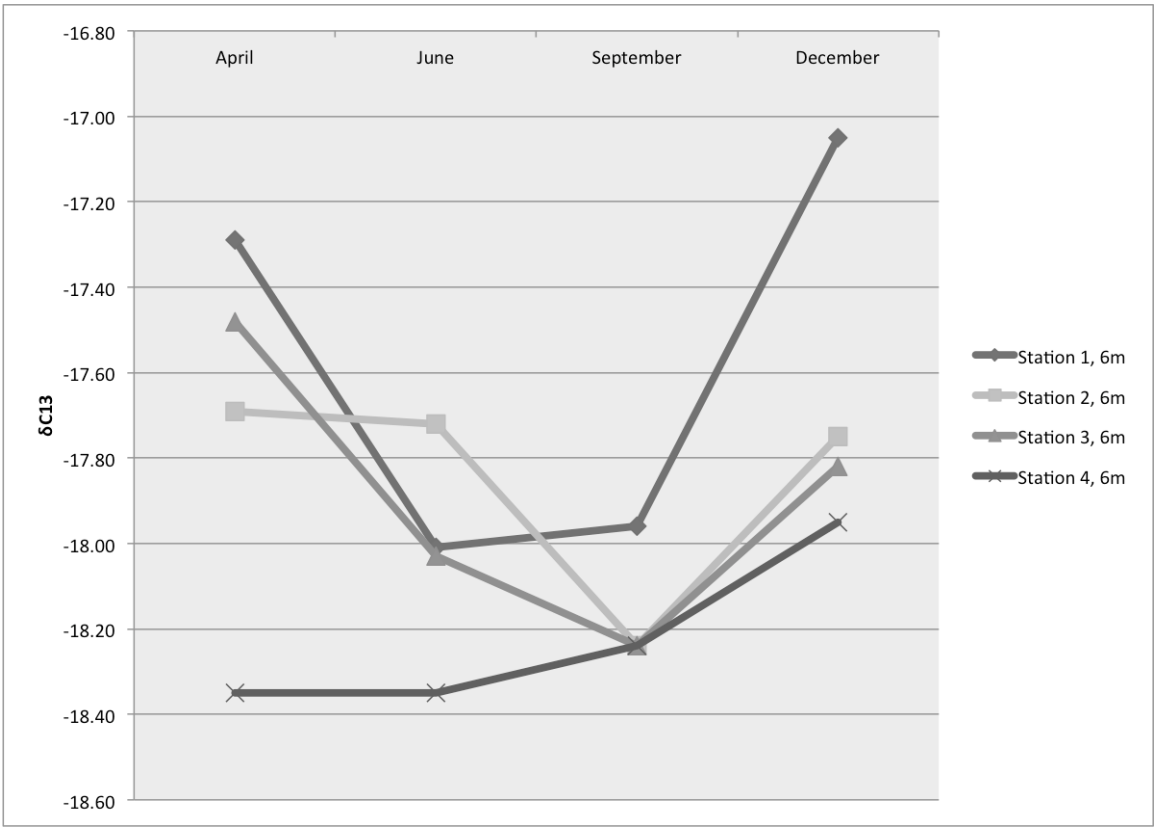

Figure 2.11. Mean δ13C values of *C. gigas* tissue samples from depths of 6m, during the sampling period (April-Dec, 2015), across all stations.

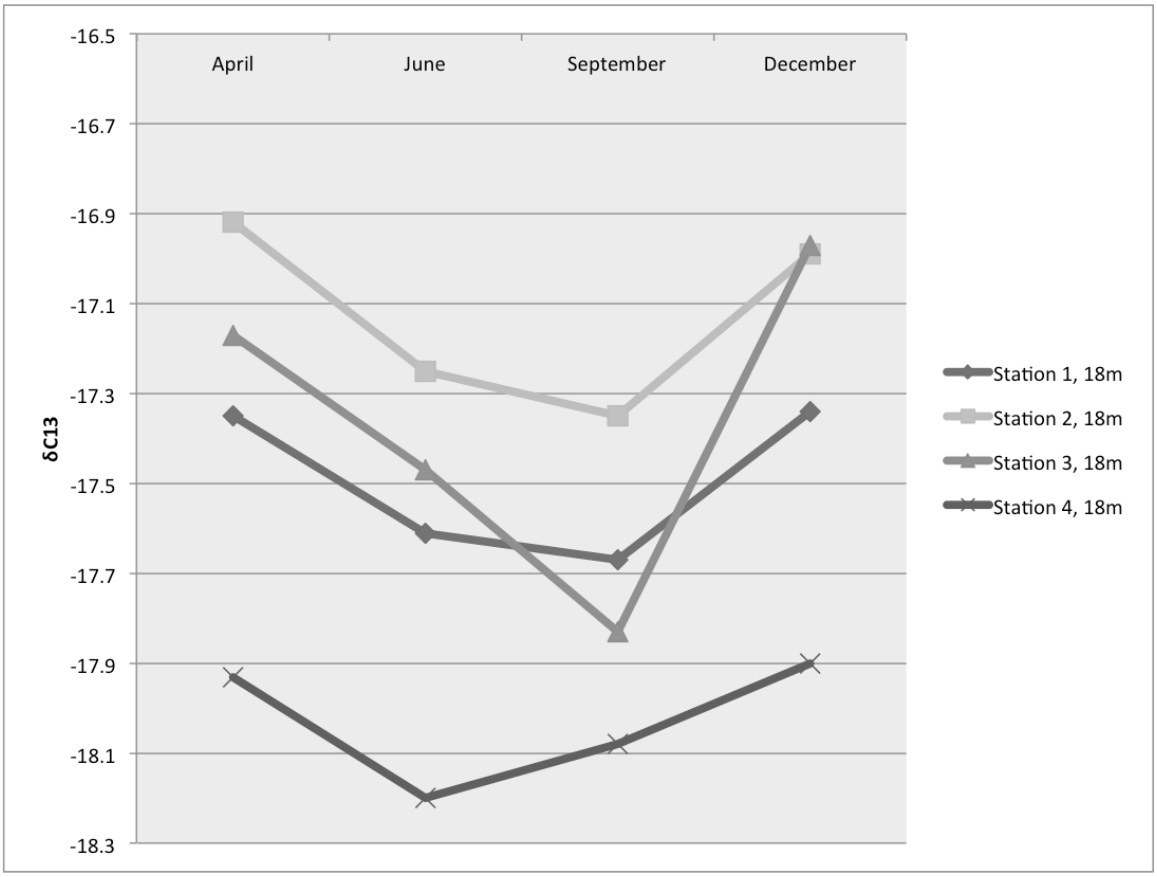

Figure 2.12. Mean δ13C values of *C. gigas* tissue samples from depths of 18m, during the sampling period (April-Dec, 2015), across all stations.

Station One	*C. gigas*, δC13			*C. gigas*, δN15		
Sample Group	Obs (*n*)	Mean	SE	Obs (*n*)	Mean	SE
Shallow (6m)						
April	5	-17.29	0.08	5	10.52	0.06
June	5	-18.01	0.06	5	10.29	0.04
September	5	-17.96	0.09	5	10.62	0.06
December	5	-17.66	0.06	5	9.62	0.05
Deep (18m)						
April	5	-17.35	0.01	5	10.54	0.02
June	5	-17.61	0.04	5	10.34	0.07
September	5	-17.59	0.08	5	10.73	0.06
December	5	-17.34	0.03	5	9.8	0.01
Fish Feed	5	-19.46	0.09	5	13.13	0.05
Feces	5	-21.15	0.06	5	8.41	0.08
POM	5	-23.83	0.08	5	7.29	0.09

Table 2.5. Station one (0m adjacent to *A. fimbria* nets) δ13C and δ15N values of *C. gigas* tissue samples, from depths of 6m and 18m, during the sampling period (April-Dec, 2015). δ13C and δ15N mean values of potential food sources included.

The station one δ13C values of *C. gigas* muscle tissue at a depth of 6m throughout the sampling period were: April, -17.29± 0.08‰; June, -18.01± 0.04‰; September, -17.96± 0.09‰; December -17.66 ± 0.06‰. The station one δN15 values of *C. gigas* muscle tissue at a depth of 6m throughout the sampling period were: April, 10.52 ± 0.06‰; June, 10.29 ± 0.04‰; September, 10.62 ± 0.06‰; December, 9.62 ± 0.05‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was used to test the significant differences among the δ13C values across months. One way ANOVA, ($F=16.35$, $p=<.05$) April vs. June (.02, $p<.05$), June vs. September ($p=.001$, $p<.01$), September vs. December ($p=.51$, $p>.05$), December vs. April ($p=.91$, $p>.05$).

The station one δ13C values of *C. gigas* muscle tissue at a depth of 18m throughout the sampling period were: April, -17.35± 0.01‰; June, -17.61± 0.04‰; September, -17.59± 0.08‰; December -17.34 ± 0.03‰. The station one δN15 values of *C. gigas* muscle tissue at a depth of 18m throughout the sampling period were: April, 10.54 ± 0.02‰; June, 10.34± 0.07‰; September, 10.73 ± 0.06‰; December, 9.80 ± 0.01‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was used to test the significant differences among the δ13C values across months. One way

ANOVA, (F=7.821 p=.0019), April vs June (p=.02, p<.05), June vs September (p=.001, p<.01), September vs December (.51, p>.05), December vs April (p=.91, p>.05).

Station Two	*C. gigas*, δC13			*C. gigas*, δN15		
Sample Group	**Obs (*n*)**	**Mean**	**SE**	**Obs (*n*)**	**Mean**	**SE**
Shallow (6m)						
April	5	-17.69	0.1	5	10.41	0.06
June	5	-17.72	0.03	5	10.14	0.09
September	5	-18.36	0.06	5	10.84	0.09
December	5	-17.75	0.07	5	10.02	0.06
Deep (18m)						
April	5	-16.91	0.04	5	9.23	0.1
June	5	-17.25	0.04	5	10.2	0.1
September	5	-17.39	0.11	5	10	0.12
December	5	-16.99	0.06	5	10.22	0.08
Fish Feed	5	-19.46	0.09	5	13.13	0.05
Feces	5	-21.15	0.06	5	8.41	0.08
POM	5	-23.83	0.08	5	7.29	0.09

Table 2.6. Station two (15m adjacent to *A. fimbria* nets) δ13C and δ15N values of *C. gigas* tissue samples, from depths of 6m and 18m, during the sampling period (April-Dec, 2015). δ13C and δ15N mean values of potential food sources included.

The station two δ13C values of *C. gigas* muscle tissue at a depth of 6m throughout the sampling period were: April, -17.69± 0.10‰; June, -17.72± 0.03‰; September, -18.36± 0.06‰; December -17.75 ± 0.07‰. The station two δN15 values of *C. gigas* muscle tissue at a depth of 6m throughout the sampling period were: April, 10.41 ± 0.06‰; June, 10.14 ± 0.09‰; September, 10.84 ± 0.09‰; December, 10.02 ± 0.06‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was used to test the significant differences among the δ13C values across months. One way ANOVA, (F= 12.72 p=.0002), April vs. June (.36, p>.05), June vs. September (.008, p<.01), September vs. December (p=.001, p<.01), December vs. April (p=.91, p>.05).

The station two δ13C values of *C. gigas* muscle tissue at a depth of 18m throughout the sampling period were: April, -16.91 ± 0.04‰; June, -17.25 ± 0.04‰; September, -17.39± 0.11‰; December -16.99 ± 0.06‰. The station two δN15 values of *C. gigas* muscle tissue at a depth of 18m throughout the sampling period were: April,

9.23 ± 0.10‰; June, 10.20 ± 0.10‰; September, 10.00 ± 0.12‰; December, 10.22 ± 0.08‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was used to test the significant differences among the δ13C values across months. One way ANOVA, (F=2.83 p=.07), April vs. June (p=.38, p>.05), June vs. September (p=.81, p>.05), September vs. December (p=.15, p>.05), December vs. April (p=.91, p>.05).

Station Three	***C. gigas*, δC13**			***C. gigas*, δN15**		
Sample Group	**Obs (*n*)**	**Mean**	**SE**	**Obs (*n*)**	**Mean**	**SE**
Shallow (6m)						
April	5	-17.48	0.05	5	10.35	0.06
June	5	-18.03	0.09	5	10.14	0.09
September	5	-18.15	0.05	5	9.04	0.07
December	5	-17.82	0.04	5	9.87	0.04
Deep (18m)						
April	5	-17.17	0.04	5	9.98	0.06
June	5	-17.47	0.05	5	10.12	0.07
September	5	-17.9	0.02	5	10.84	0.04
December	5	-16.97	0.03	5	10.01	0.04
Fish Feed	5	-19.46	0.09	5	13.13	0.05
Feces	5	-21.15	0.06	5	8.41	0.08
POM	5	-23.83	0.08	5	7.29	0.09

Table 2.7. Station three (30m adjacent to *A. fimbria* nets) δ13C and δ15N values of *C. gigas* tissue samples, from depths of 6m and 18m, during the sampling period (April-Dec, 2015). δ13C and δ15N mean values of potential food sources included.

The station three δ13C values of *C. gigas* muscle tissue at a depth of 6m throughout the sampling period were: April, -17.48± 0.05‰; June, -18.03± 0.09‰; September, -18.15± 0.05‰; December -17.82 ± 0.04‰. The station three δN15 values of *C. gigas* muscle tissue at a depth of 6m throughout the sampling period were: April, 10.35 ± 0.06‰; June, 10.14 ± 0.09‰; September, 9.04 ± 0.07‰; December, 9.87 ± 0.04‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was used to test the significant differences among the δ13C values across months. One way ANOVA, (F=33.7 p<.01), April vs. June (p=.001, p<.01), June vs. September (p=.64, p>.05), September vs. December (p=.001, p<.01), December vs. April (p=.008, p<.01).

The station three δ13C values of *C. gigas* muscle tissue at a depth of 18m throughout the sampling period were: April, -17.17± 0.04‰; June, -17.47± 0.05‰; September, -17.90± 0.02‰; December -16.97 ± 0.03‰. The station three δN15 values of *C. gigas* muscle tissue at a depth of 18m throughout the sampling period were: April, 9.98 ± 0.06‰; June, 10.12 ± 0.07‰; September, 10.84 ± 0.04‰; December, 10.01 ± 0.04‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was used to test the significant differences among the δ13C values across months. One way ANOVA, (F= 54.76 p=<.01), April vs. June (p=.31, p>.05), June vs. September (p=.001, p<.01), September vs. December (p=.001, p<.01), December vs. April (p=.09, p>.05).

Station Four (reference)	***C. gigas*, δC13**			***C. gigas*, δN15**		
Sample Group	**Obs (*n*)**	**Mean**	**SE**	**Obs (*n*)**	**Mean**	**SE**
Shallow (6m)						
April	5	-18.85	0.07	5	10.73	0.13
June	5	-18.24	0.03	5	10.28	0.07
September	5	-18.24	0.05	5	10.84	0.11
December	5	-17.99	0.01	5	10.02	0.04
Deep (18m)						
April	5	-17.93	0.02	5	9.62	0.08
June	5	-18.3	0.1	5	9.99	0.06
September	5	-17.78	0.05	5	10.6	0.13
December	5	-17.9	0.12	5	9.98	0.11
Fish Feed	5	-19.46	0.09	5	13.13	0.05
Feces	5	-21.15	0.06	5	8.41	0.08
POM	5	-23.83	0.08	5	7.29	0.09

Table 2.8. Station four (reference) δ13C and δ15N values of *C. gigas* tissue samples, from depths of 6m and 18m, during the sampling period (April-Dec, 2015). δ13C and δ15N mean values of potential food sources included.

The station four δ13C values of *C. gigas* muscle tissue at a depth of 6m throughout the sampling period were: April, -18.85± 0.07‰; June, -18.24± 0.03‰; September, -18.24± 0.05‰; December -17.99 ± 0.01‰. The station one δN15 values of *C. gigas* muscle tissue at a depth of 6m throughout the sampling period were: April, 10.73 ± 0.13‰; June, 10.28 ± 0.07‰; September, 10.84 ± 0.11‰; December, 10.02± 0.04‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was

used to test the significant differences among the δ13C values across months. One way ANOVA (F=26.1 p<.05), April vs. June (p=.001, p<.01), June vs. September (p=.91, p>.05), September vs. December (p=.91, p>.05), December vs. April (p=.001, p<.01).

The station four δ13C values of *C. gigas* muscle tissue at a depth of 18m throughout the sampling period were: April, -17.93± 0.02‰; June, -18.30± 0.10‰; September, -17.78± 0.05‰; December -17.90 ± 0.12‰. The station four δN15 values of *C. gigas* muscle tissue at a depth of 18m throughout the sampling period were: April, 9.62 ± 0.08‰; June, 9.99 ± 0.06‰; September, 10.6 ± 0.13‰; December, 9.98 ± 0.11‰. Analysis of variance (one-way ANOVA) with the Tukey HSD *post hoc* test was used to test the significant differences among the δ13C values across months. One way ANOVA (F= 2.4, p=.11), April vs. June (p=.24, p>.05), June vs. September (p=.10, p>.05), September vs. December (p=.91, p>.05), December vs April (p=.91, p>.05).

2.5 Discussion

2.5.1 Spatial

IMTA is based on an ecological approach to minimizing the impact of aquaculture activities on marine food webs, which is maximized by the strategic placement of both organic and inorganic extractive species (Cross, 2004). The primary objective of the present study was to assess the effect of spatial factors on the assimilation of aquaculture-derived waste from a Sablefish (*A. fimbria*) into the diet of Pacific oysters (*C. gigas*). Many studies have established the ability of filter feeding bivalves to consume aquaculture-derived waste, through laboratory studies and through in-situ research (Orr, 2011; Chopin, 2010; Cross, 2004). By examining the relationship between spatial placement of filter feeding bivalves, and the influence of aquaculture waste on their diet, it is possible to develop a greater understanding of ideal placement for filter feeding bivalves within an IMTA system.

The isotope dataset showed that *C. gigas* in certain stations were more effective at assimilating caged-fish-derived organic waste into their diet, at a depth of 18m of in particular. This result supports the conclusion that strategic placement of extractive organic species within an IMTA system contributes to a reduction in sedimentary loads of organic waste, and thereby to an improvement in sediment and water quality through

the extractive process.

Feces of cage-cultured fish had isotope signatures that were distinguishable (more enriched δ13C but more depleted δ15N) from those of the fish feeds (-19.46± 0.09‰ vs. -21.15 ± 0.06‰; 8.41 ± 0.08‰ vs. 13.13 ± 0.05‰, respectively). Such an isotopic discrepancy between feed and feces reflects differences in metabolic activity of fish species and composition of their feed (McGhie et al., 2000; Franco-Nava et al., 2004). Yokoyama et al. (2006) reported that cultured fish, which fed on artificial feeds, excrete feces containing specific undigested materials, which contribute to an inequality in isotopic signatures of feed and feces. Many studies also report no discrepancy between feed and feces (Mazzola & Sarà 2001; Ye *et al.* 1991; Sutherland *et al*. 2001), which suggests that this relationship is dependent upon the type of feed and species of fish. Despite the considerable δ15N depletion in feces of the cultured Sablefish compared with that of the fish feeds, both the feces' δ13C and δ15N signatures were significantly different from those of naturally occurring POM (-21.15 ± .06‰ vs -23.83 ± .08‰; 8.41 ± .08‰ vs 7.29 ± .09‰ respectively).

The April δ13C values of muscle tissue from *C. gigas* suspended at a depth of 6m at all locations (stations 1-3) within the IMTA system were significantly different from those of the reference site oysters ($F_{3,16}$=78.96, p=<.05), suggesting that all oysters within the IMTA system consumed aquaculture-derived waste as a major part of their diet. Station one exhibited the most enriched values (-17.29 ± 0.08‰), station three δ13C values were the second most enriched (-17.48 ± 0.05‰), with station two exhibiting the least enriched values (-17.69 ± 0.10‰). Within the IMTA system, stations two, three, and four did not show any significant difference from one another [(station one vs station three (p=.36, p>.05), station two vs station three (p=.28, p>.05)]. These results suggest that oysters at all locations at a depth of 6m within the IMTA system consumed primarily aquaculture waste, while oysters at the reference site consumed primarily POM. The lack of significant differences between the stations within the IMTA system suggest that all stations consumed aquaculture waste evenly, with little to no spatial variation.

The April δ13C values of muscle tissue from *C. gigas* suspended at a depth of 18m at all locations (stations 1-3) within the IMTA system were very significantly different from those of the reference site oysters ($F_{3,16}$=167.449, p=<.01), as well as from

each other, indicating a strong influence of spatial variation on oyster diets [station one vs station two ($p=.001$, $p<.01$), station one vs station three ($p=.01$, $p<.05$), station one vs station four ($p=.001$, $p<.01$), station two vs station three ($p=.001$, $p<.01$), station two vs station four ($p=.001$, $p<.01$), station three vs station four ($p=.001$, $p<.01$)]. Reference site oysters were significantly less enriched (-17.93 ± 0.02‰) than all stations within the IMTA system, suggesting that their diet consisted primarily of POM, while IMTA oysters all consumed primarily aquaculture waste. However, all stations within the IMTA system showed significant differences from one another, suggesting that each station consumed waste in different quantities. Station two showed the least enriched values (-16.91 ± 0.04‰), which suggests the greatest assimilation of aquaculture waste. Station three exhibited the second least enriched values (-17.17 ± 0.04‰) suggesting both the presence and subsequent uptake of waste at a depth of 18m, traveling away from the fish nets, with a stronger signal closer to the nets. Station one exhibited the most enriched values within the IMTA system (-17.35± 0.01‰) suggesting the least influence of aquaculture waste in the oysters' diets within the IMTA system. This indicates that station one may be out of the main waste plume. Differences between stations in the month of April were the strongest at 18m, than at any depth in any other month of the year, suggesting that oysters within the IMTA system feed the most heavily on aquaculture waste at this time and at this depth.

The δ13C values of *C. gigas* muscle tissue at a depth of 6m in June showed no significant differences between any stations ($F_{3,16}=1.23$, $p>.05$). Station one vs station two ($p=.88$, $p>.05$), station one vs station three ($p=.90$, $p>.05$), station one vs station four ($p=.69$, $p>.05$), station two vs station three ($p=.51$, $p>.05$), station two vs station four ($p=.90$, $p>.05$), station three vs station four ($p=.32$, $p>.05$). δ13C values were all consistent with expected values for oysters primarily feeding on POM. These results suggest that the abundance of POM in the water column, at 6m of depth, during this time period, replaces aquaculture-derived waste as the main source of food for oysters within the IMTA system. The data suggest that there was no influence of spatial variation on the diet of the oysters during this month.

In contrast to the oysters placed at a depth of 6m, the δ13C values of *C. gigas* muscle tissue at a depth of 18m in June showed very significant differences between

stations ($F_{3,16}$=29.13, p<.01), with the exception of stations two and three. In fact, the only month where stations two and three differed significantly from one another at any depth was at 18m during April. These results suggest that there is no difference in uptake of aquaculture derived waste between stations two and three during most of the year. All δ13C values of *C. gigas* muscle tissue from stations within the IMTA system showed significant differences from those of the reference oysters [station one vs station four (p=1.52, p>.05) station two vs station four (p=.51, p>.05), station three vs station four (p=2.51, p>.05)]. Consistent with results from April, station one exhibited the least enriched δ13C values of *C. gigas* muscle tissue, suggesting that the influence of aquaculture-derived waste within the IMTA system had the least impact on this station (-17.61± 0.04‰). However, the presence of aquaculture-derived waste in the oysters' diets was still present, as this station's δ13C values were significantly less enriched than reference oysters. δ13C values of oyster tissue from station two exhibited the strongest influence of aquaculture waste in the diet. Consistent with April results, these data suggest that aquaculture-derived waste is travelling through station two, then passing through station three which exhibited slightly less enriched δ13C values (-17.47± 0.05‰). Oyster at station two exhibit δ13C values most consistent with a diet of primarily aquaculture waste, followed by station three, with station one as the least impacted within the IMTA system.

The δ13C values of *C. gigas* muscle tissue at a depth of 6m in September showed some significant differences between stations ($F_{3,16}$=11.74, p=<.05). However, all tissue showed less enriched values, indicating POM as an important food source for oysters during this time period, [(station one (-17.96 ± 0.09‰; station two, -18.36 ± 0.06‰; station three, -18.15 ± 0.05‰; station four (reference) -18.24 ± 0.05‰)]. Interestingly, during this time period, station one exhibits the most enriched δ13C values, indicating that aquaculture-derived waste is still present in the diet. At all other stations, less enriched values combined with insignificant differences between mean δ13C values suggest that oysters at this depth during this time period are feeding primarily on POM.

The δ13C values of *C. gigas* muscle tissue at a depth of 18m in September were less enriched overall than would be expected for a diet composed of entirely aquaculture-derived waste. Station one exhibited the most enriched δ13C values, indicating that

oysters fed on aquaculture-derived waste in this location, in significantly greater quantities than at any other station. Station one is the only station that exhibited a significant difference in δ13C values from the reference station [station one vs station four (p=.04, p<.05)], suggesting that stations two and three consumed as much POM as oysters at the reference station [station two vs station four (p=.22, p>.05), station three vs station four (p=.91, p>.05)].

The δ13C values of *C. gigas* muscle tissue at a depth of 6m in December within the IMTA site showed some significant differences from reference site values (-17.99 ± .01%, $F_{3,16}$=8.25, p=<.01). Stations one and two were significantly more enriched than reference site oysters [(station one, -17.66± 0.06‰; station two, -17.75 ± 0.07‰, station one vs station four (p=.001, p<.01) station two vs station four (p=.02, p<.05)] suggesting the strong influence of aquaculture-derived waste on the oysters' diets in these locations. However, station three did not show any significant difference from reference site oysters, suggesting that this station fed primarily upon POM during this time (station three vs station four (p=.10, p>.05).

The December δ13C values of *C. gigas* muscle tissue at a depth of 18m showed a strong significant difference between all stations within the IMTA system and reference site oysters [$F_{3,16}$=62.79, p=<.05; station one vs station four (p=.001, p<.01), station two vs station four (p=.001, p<.05), station three vs station four (p=.001, p<.05)], suggesting that aquaculture-derived waste was the primary source of nutrients for all stations within the IMTA system at all stations during this month. Station one was the least enriched (-17.34 ± 0.03‰) indicating a weaker presence of aquaculture-derived waste within the oysters' diets. Station two and station three showed no significant difference from one another (station two vs station three, p=.91, p>.05), and were the most enriched, indicating a greater reliance upon aquaculture-derived waste as a source of nutrients.

The results from this study support the concept of culturing pacific oysters in close proximity to fish cages in IMTA systems as a means to absorb and transform solid waste organic nutrients. This study also suggests that simply placing oysters arbitrarily will not always ensure contact of the oysters with a consistent 'stream' of farm particulates for ingestion. Consequently, one of the major design challenges to IMTA stations will be, understanding particulate plume dynamics exiting cages so that the

placement of co-cultured suspension or filter feeders will have maximum access.

In Kyuquot sound, at Kyuquot SEAfoods ltd, it is apparent that oysters suspended at a depth of 18m were overall more effective at filtering aquaculture-derived waste from the water column. These effects were strongest in the months of April and December. Oysters suspended at a depth of 6m showed more variability in diet composition, but also exhibited tendencies to feed on aquaculture derived waste throughout the year. The isotopic dataset provides evidence of the pacific oyster's suitability as an extractive species that can be used to reduce the impact of an intensive monoculture system on the ambient environment. The dataset also suggests that stable isotope analysis may act as a quick tool to examine the relationship between extractive organism placement and diet composition.

2.5.2 Temporal

δ13C values of *C. gigas* muscle tissue sampled from a depth of 6m at station one varied significantly throughout the course of the sampling period ($F=16.35$, $p=<.05$). Values were significantly less enriched during the month of June (-18.01±.06‰), and September (-17.96±.09‰) compared to April and December values (-17.29±.08‰ and -17.66±.06‰ respectively, April vs. December $p=.91$). These results indicate that oysters compensated for a lack of POM in their diet by consuming greater quantities of aquaculture-derived effluent during the winter months. At a depth of 18m, however, June was the only month to exhibit significantly different, and less enriched, δ13C values (-17.61± 0.04‰) compared to all other sampling months. These results indicate consistent consumption of aquaculture-derived effluent by *C. gigas* at this depth throughout the course of the year, with an increase in POM consumption only during a brief period over the summer.

The station two δ13C values of *C. gigas* muscle tissue at a depth of 6m throughout the sampling period were significantly similar during the months of April, June, and December (April, -17.69± 0.10‰; June, -17.72± 0.03‰; December -17.75 ± 0.07‰). September δ13C values were significantly less enriched than all other months in the sampling period (-18.36± 0.06‰), indicating a significant increase in the quantity of

marine POM consumed by *C. gigas* at this depth during this time. Results indicate a consistent reliance by *C. gigas* upon aquaculture-derived effluence as a source of nutrition over the course of the year, with an increase in marine POM consumption during only a brief period of time in the late summer. The station two δ13C values of *C. gigas* muscle tissue at a depth of 18m were the only values within the IMTA system in this study to remain statistically similar throughout the course of the entire sampling period. Values were enriched throughout the course of the year (April, 10.41 ± 0.06‰; June, 10.14 ± 0.09‰; September, 10.84 ± 0.09‰; December, 10.02 ± 0.06), with no significant variation in diet composition. These results indicate a strong reliance upon aquaculture-derived effluent as a source of nutrition, on an annual basis. Marine POM did not appear to act as a source of nutrition for *C. gigas* at this station at any point during the year.

The station three δ13C values of *C. gigas* muscle tissue at a depth of 6m varied significantly on a monthly basis throughout the sampling period. June and September δ13C values were statistically similar, while April and December values were significantly different from all other months. June and September exhibited less enriched values than during other months (June, -18.03± 0.09‰; September, -18.15± 0.05‰), while December and April were slightly more enriched (December, -17.82 ± 0.04‰, April -17.48± 0.05‰). These results indicate an increased reliance upon aquaculture-derived effluence as a nutritient subsidy during the months of April and December, though the numbers throughout the year are overall less enriched than some other stations. These results indicate that oysters at this depth relied upon marine POM as a primary source of nutrition throughout the sampling period, though aquaculture-derived effluent was also consumed during the months of April and December. The station three δ13C values of *C. gigas* muscle tissue at a depth of 18m throughout the sampling period exhibited significant differences form one another. September was significantly less enriched than all other months (-17.90± 0.02‰) indicating a strong reliance upon marine POM as a source of nutrients. April and December exhibited the most enriched values (April, -17.17± 0.04‰, December -16.97 ± 0.03‰) indicating the influence of aquaculture-derived effluent on the diet of *C. gigas* at this station during these months. June differed significantly from December values ($p<.01$), but not from April values

(p=.31), indicating that oysters relied upon both marine POM and aquaculture-derived effluent as sources of nutrients during this time.

The station four δ13C values of *C. gigas* muscle tissue at a depth of 6m throughout the sampling period were significantly consistent, with the exception of the month of April. These results indicate that oyster feeding habits did not change over the course of the year, but relied heavily upon marine POM as a source of nutrients on an annual basis. However, δ13C values for the month of April were significantly less enriched than values during all other months (April, -18.85± 0.07‰) indicating a strong reliance upon marine POM during this time. Results indicate that marine POM signatures may change throughout the year, or that an additional naturally occurring food source may be available to oysters at this depth at certain times. Further sampling would be necessary to elucidate these findings. The station four δ13C values of *C. gigas* muscle tissue at a depth of 18m showed no significant differences throughout the sampling period. These results indicate that oysters at the reference site, at this depth, fed consistently upon marine POM throughout the course of the year. These results are in line with predictions that oysters feeding upon marine POM would exhibit consistent feeding patterns throughout the course of the year. These results, however, only indicate percent composition of the oysters' diets, without providing information regarding the actual amount that oysters consumed on a monthly basis. These results combined with growth data would be useful, in order to indicate whether or not the amount of marine POM consumed changed over the course of the year.

In this study, results show that over the course of one year, isotopic values within C. gigas muscle tissue at all stations within an IMTA system were not consistently enriched compared to those taken at a reference site. These results indicate the tendency of oysters to feed preferentially upon marine POM when available. The influence of marine POM on the diets of C. gigas was not uniform across stations within the IMTA facility, indicating that marine POM was not available in similar quantities to all stations. Stations where values were consistently enriched over the course of the year indicate either preferential feeding upon IMTA-derived effluent, or a lack of marine POM. Results show that oysters compensate for the lack of marine POM during winter months, as well as possible patchiness within the water column, by consuming aquaculture-

derived waste in the form of both feces and feed pellets. These results are consistent with research conducted on *C. gigas* feeding patterns, related to marine phytoplankton productivity.

In a study of farmed Pacific oysters *C. gigas*, Brown (1988) measured the growth rate in shell height over a 14-month period in ten different locations in British Columbia. Values were compared to physical environmental parameters (salinity, total particulate matter, particulate organic matter, particulate inorganic matter, carotenoids, chlorophylls *a*, *b*, and *c* concentrations, pH, dissolved oxygen, and water temperature). It was found that instantaneous growth in *C. gigas* was strongly correlated with chlorophyll *b*, which is indicative of the influence of marine plankton to their diets. Highest concentrations of chlorophyll *b* occurred during the summer months, which correlated with highest shell growth rates of *C. gigas*. Isotopic values were significantly more enriched during the fall and winter months, when chlorophyll *b* levels, total particulate matter, and water temperature are relatively low in this region.

The selective feeding habits of *C. gigas* may account for the temporal variability in dominant food sources demonstrated by the results. Overall, the monthly fluctuations of trace carbon and nitrogen signatures demonstrated in this study are likely characterized by the availability of marine POM. As the study approached summer, δ13C signatures of most samples studied became less enriched, possibly owing to an increasing production of marine phytoplankton. Signatures remained less enriched until fall sampling dates, after which they remained consistently enriched throughout the winter.

The findings of this paper are consistent with literature describing increased growth rates of shellfish cultured within IMTA systems. While oysters may not consume aquaculture-derived effluent at consistent rates throughout the year, results indicate that seasonal and spatial deficiencies in the availability of marine POM are compensated for with increased consumption of aquaculture-derived effluent.

3.0 Conclusions

Food webs are useful in that they provide a conceptual structure for assessing the importance of consumer-resource trophic relationships in ecosystems. The increased use of natural stable isotopes as tracers of consumer-resources trophic links demonstrates its utility as a powerful tool in marine food web analysis. Marine communities are ideal research models in that physical and biological interactions occur over numerous spatial-temporal scales. Integrated multi-trophic aquaculture systems are designed using principles that govern naturally occurring marine food webs, in order to create a sustainable farming operation. By examining trophic relationships within an IMTA system, it is possible to map the flow of nutrients from one component to another.

Analyzing organic extractive organisms within an integrated multi-trophic aquaculture (IMTA) facility offered an opportunity to better understand the influence of aquaculture-derived organic effluent as a potential nutrient subsidy. This system currently cultures three extractive species, representing both marine producers and consumers: sablefish *Anoplopoma fimbria*, Pacific scallops *Patinopectin caurinus*, and sugar kelp *Saccharina latissima*. In all IMTA facilities, extractives are chosen based on documented husbandry practices, habitat suitability, biomitigation capacity, and economic value. IMTA stations are spatially manageable and abundantly reared populations of extractives offer an opportunity for extensive research. Furthermore, residency of aquaculture facilities in one location permits longer term temporal examination of community dynamics, offering ideal experimental conditions.

This thesis represents my investigation into the influence of IMTA organic effluent waste on intended extractive species within the facility. I used stable carbon and nitrogen isotope analysis to establish trophic linkages between extractive organisms, and the fed component within the IMTA facility. I acquired data over the course of one year in order to assess possible monthly fluctuations in isotopic signatures. I made the following predictions:

1) *Crassostrea gigas* species sampled within the IMTA site would have enriched carbon and nitrogen isotopic signatures that, if assimilated, would reflect the organic waste subsidy expected from a finfish farming operation. Furthermore, these signatures should

be more enriched than those found within the tissues of the same species sampled at the reference site.

2) Temporal fluctuations in signatures among IMTA site sampled species would be minimal as aquaculture derived organic effluent is considered a constant potential subsidy.

3) *Crassostrea gigas* species sampled at depths of 6m and 18m, and at distances of 0m, 15m, and 30m from the fed component, within the IMTA site, would exhibit significantly different isotopic signatures from one another. This would indicate spatial variability in the presence, and subsequent uptake, of aquaculture-derived waste within the IMTA system.

Enriched isotopic signature results for pacific oysters *Crassostrea gigas* sampled at the IMTA facility agreed with my first prediction. However, results were not uniform across seasons, violating my third assumption. Temporal results showed that the overall uptake of aquaculture-derived waste was greatest during the winter and spring months (December and April), and least significant during the summer and fall months (June and September). Potential causes for this disparity may be related to the availability of marine POM as a source of nutrition for oysters. Because of their ability to feed selectively, it appears that they select naturally occurring food sources over IMTA derived effluent. Results from this study indicate that oysters compensate for any reduction in the availability of marine POM, by substituting aquaculture-derived waste into their diets. However, during the summer months, isotopic values of C. gigas muscle tissue within the IMTA system exhibited some significant differences from the reference site, indicating that aquaculture-derived waste continues to be an important part of oysters' diets throughout the year.

Assumption two was supported by results showing significant differences between isotopic signatures of oysters at different depths and at distances from the fed component, within the IMTA system. Results suggest that the availability of aquaculture-derived effluent is not uniform within the water column, and is subject to variability. Overall, however, deeper stations (18m) placed further away (15 and 30m) from the fed

component exhibited the most consistently enriched values, indicating the strongest presence and subsequent uptake of aquaculture-derived effluent by oysters at those stations.

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