Spatial-temporal influence of integrated multi-trophic aquaculture-derived organic effluent on adjacent marine communities

by

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Supervisory Committee

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Abstract

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Aquaculture facilities have been demonstrated to emit massive quantities of waste that incorporates in to the surrounding water column, effectively altering patterns and processes of nearby marine communities. Given that products from aquaculture is heavily relied upon to meet global fisheries demands, understanding its effects is essential to inventing less harmful practices. This research examines one such facility located in Kyuquot, British Columbia. The purpose of this thesis is to spatially and temporally measure the degree and magnitude of integrated multi-trophic aquaculture (IMTA)-derived organic waste as a potential subsidy to adjacent marine communities. Stable carbon and nitrogen isotopes analysis was applied to intended extractive organisms (sablefish *Anoplopoma fimbria*, Pacific scallops *Patinopectin caurinus*, blue mussels *Mytilus edulis*, sea urchin *Strongylocentrotus franciscanus*, sea cucumber *Parastichopus californicus*, kelp *Saccharina latissima*), epibiont biofouling species (brooding transparent tunicates *Corella inflata*, hairy tunicate *Boltenia villosa*, broadbase tunicates *Cnemidocarpa finmarkiensis*) as well as fish feed and sablefish faeces. Stable isotopes of blue mussels and brooding transparent tunicates sampled from both the IMTA and a reference site were compared in order to examine spatial influence of IMTA-derived waste. IMTA site sampled mussels exhibited the most enriched and least variable values among all four sample groups. Brooding transparent tunicates exhibited the most isotopic variability which demonstrates that IMTA-derived waste is not among the most important food source available. This is corroborated by the three-source mixing model results. Only sablefish isotopic signatures were measured to be more enriched than those of fish feed and fish faeces. Isotopic mixing models were employed to all IMTA samples and found that IMTA effluent signatures were proportionately higher in their diets than

averaged marine particulate organic matter (POM) signatures taken from the literature. Mixing model results also showed IMTA effluent to be proportionately less than marine POM. Circular statistical results did not demonstrate particular directional change for all IMTA sampled isotopic signatures which could be due to the consistent nature of available fish feed throughout the year and/or perhaps feeding choice changes constantly. Further examination into the monthly physical properties of this region (eg. rainfall, irradiance) as well as measurements of marine POM signatures would greatly compliment these results and are recommended for future study.

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Co-Authorship Statement

This thesis is an amalgamation of two scientific manuscripts of which I am lead author. The general idea involving stable isotopic analysis of organic extractives within an integrated multi-trophic aquaculture project was proposed by Dr. Dennis E. Jelinski, who had identified this project as being a unique research opportunity. I performed all of the research, data collection and analysis, interpretation of results, and preparation of final manuscripts. Dr. Stephen F. Cross provided assistance with facility and equipment usage, and transportation. Dr. Jelinski provided editorial suggestions wherever needed.

1.0 GENERAL INTRODUCTION

1.1 Research context

Species that make up ecological communities are often depicted in the form of a food web, where predator and prey occupy variable positions in trophic niche space and time linked through energy transfers (Elton 1927; Paine 1980; Pimm 1982; Paine 1988; Polis 1991). 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 (Hairston *et al*. 1960; Oksanen *et al*. 1981). Contemporary theory conceptualizes food webs as highly complex and spatially heterogenous; models contain thousands of species connected via multiple and variably strengthened linkages with consumption and productivity spatially existing in a multitude of directions throughout the food web spectrum (Polis & Strong 1996; Polis *et al*. 1997). Linear models are, according to Polis & Strong (1996), unable to adequately accommodate for dynamics of detritus, omnivory, spatial resource subsidies across habitats, looping or nutrients. Despite oversimplification for scientific utility in trophic depictions, the classic food chain model continues to provide the basis for most food web studies today (Dunne *et al*. 2004).

Marine food webs and primary productivity are generally determined by multi-directional 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 nutrient subsidies across habitats. These nutrients include faecal matter rich in fertilizing nitrogen useful to bottom dwelling detritivore communities. Dissolved ammonia and other inorganic compounds found in fish faeces promote growth in phytoplankton and macrophytes throughout the photic

zone of the water column (Pandian 1976; Folke & Kautsky 1989; Coen & Neori 1991). Primary producers are essential for secondary production, thereby marking nutrient subsidies as being vital in shaping diversity, density and biomass of species within food webs (Vizzini & Mazzola 2003). Overproduction of phytoplankton due to higher than normal concentrations of particulate organic and inorganic matter by-products can stimulate conditions that consequently lead to anoxia and eutrophication (Troell & Berg 1997).

Many studies report negative effects of elevated loading of aquaculturally derived fish waste to adjacent marine communities (Gowen & Bradbury 1987; Wu *et al*. 1994; Ervik *et al*. 1997; Karakassis et al. 2000; Naylor *et al*. 2000; Gao *et al*. 2006). Aquaculturally generated discharge is high in nitrogen and phosphorus concentrations dissolved from waste feed and fish faeces, consequently altering adjacent biotic communities (Parsons *et al*. 1977; Folke & Kautsky 1989; Lin 1989; Silvert 1994; Levings 1997). Su *et al*. (1993) determined that high nutrient loadings generated by aquaculture systems supported blooms of red tide forming *Alexandrium tamarense*, a toxic dinoflagellate responsible for mass mortalities in southern Taiwan shrimp ponds. Mazzola *et al*. (1999) measured the initial impact of nutrient loadings directly below fish cages and found increased levels of accumulated biopolymeric carbon 6 weeks after installment of fish cage structures. Significant decreases in density of meiofaunal assemblages directly below fish cages were traced to these sedimental changes.

In addition to ecological effects created by traditional monoculture systems, negative social and economical impacts, such as loss of natural goods and services, mark a heightened need for change (Primavera 2006). Despite its negative impacts, the gains from some aquaculture industries outweigh those from the catch fisheries (Bardach 1986). Folke & Kautsky (1991) compared various fisheries and aquaculture systems and determined one-species aquaculture systems to exhibit comparable characteristics to those of stressed natural ecosystems. They proposed increase development of more efficient cultivation methods; modeling Chinese integrated systems based on ecological engineering principals that mimic natural

ecosystems and require less resource input. Polycultures attempt to rear harvestable organisms of compatible feeding levels Bardach 1986), but without regard for species representing different trophic levels.

Integrated multi-trophic aquaculture (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 via water currents and gravity (Chopin *et al*. 2001; Neori *et al*. 2004). Within these systems, uneaten fish feed and fish faeces are transferred to other co-culturedspecies 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 blue mussels (*Mytilus edulis*), 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). Sarà *et al*. (2009) 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.

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 (eg. with kelp, sea otters and sea urchins, Estes & Palmisano 1974; Duggins 1980), stomach content analysis (eg. with shelf ecosystems, Link 2002; and amphipods, Marion *et al*. 2008), radio-tracer techniques (eg. in coral reef and subtropical estuaries, Smith *et al*. 1979; and bivalve molluscs, Wang & Fisher 1999) and natural stable isotopes analyses (Michener & Schell 1994; 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 that can challenge the ability to obtain an adequate prey sampling. Gut content analyses can be time consuming; requiring extensive specimen collection and dissection of content that will likely only

represent a "snapshot" of a consumer diet (Schindler & Lubetkin 2004). Moreover, organisms digest their prey at varying rates, which can prove challenging for identification purposes when content becomes less intact or recognizable over time (Michener & Schell 1994). Radio-tracer techniques involve artificially and uniformly labeling a potential prey with an isotope (eg. ^{14}C), then releasing the labeled prey, and the uptake or loss of the isotope is measured 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. Stable isotope analysis applied to ecosystem questions are most widely used because isotope data can provide both source-sink and process information (Peterson & Fry 1987). Data acquisition for stable isotopes is straightforward; sampling of a subset food web population can be simple and the analytical technology is advanced.

All of the contents within the Earth and its atmosphere are made up of different elements, including carbon, oxygen, nitrogen, sulfur and hydrogen. Based on their atomic weights, each of these elements exists in different forms. The majority of carbon exists as carbon 12 (^{12}C), but approximately one percent can be found in the heavier form of carbon 13 (^{13}C). In the biosphere, the ratio of both stable carbon forms ($^{12}C/^{13}C$) is equal. As plants take in carbon atoms, water, and soil from the atmosphere, the process of photosynthesis alters the ratio while being stored. This altered ratio, however, remains stable and relatively unchanged throughout its passage through the food web (DeNiro & Epstein 1978; Peterson & Fry 1987). This generalized theory holds true for other atoms.

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

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3$$

where δ is the sample isotopic measurement notation (heavy to light atomic weight ratios), X equals ^{13}C and ^{15}N, and R corresponds to the ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$.

Changes in the δ value reflect ratio differences in heavy to light isotopic values. For instance, as $\delta^{15}N$ increases in a sample, the proportion of ^{15}N (heavy) increases, while the proportion of ^{14}N (light) decreases (Peterson & Fry 1987). δ values are then referenced to known standard materials, depending on the isotope analyzed (this is 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; dissected sample tissues must first be ground to a powder and converted to pure gas (CO_2 and N_2). 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 only a 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—approximately 3 to 4 ‰ enrichment relative to prey (DeNiro & Epstein 1978; Frederiksen 2003). The ^{15}N enrichments in animal isotopic versus dietary composition are mainly due to the excretion of ^{14}N in urine, which is ^{15}N depleted. Nitrogen isotopic ratio values for faeces 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 (Michener & Schell 1994; Vizzina & Mazzola 2002).

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 (Ye *et al*. 1991; McGhie *et al*. 2000; Mazzola & Sarà 2001; Sarà *et al*. 2004; Dubois *et al*. 2007). Gao *et al*. (2006) used the carbon and nitrogen stable isotope approach and determined enrichment of signatures among green-lipped mussels *Perna viridis* 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 $\delta^{15}N$ signatures within epiphyte and seagrass *Posidonia oceanica* leaf tissues adjacent to a large fish farm facility. At present, there is a dearth of research that has utilized stable isotope analysis to elucidate trophic linkages between extractive species within an IMTA facility.

1.2 Research focus

Nitrogen is often the limiting nutrient in the majority of marine environments (Brooks & Mahnken 2003). Along the west coast of North America, relatively cold, upwelled, marine waters are particularly productive, often high in nitrate, a particularly important source of nitrogen to the euphotic zone (Michener & Schell 1994). These nutrient-rich waters get replaced by surface waters that get pushed offshore by northwesterly winds by the Coriolis effect. A large proportion of phytoplankton are forced below compensation depth by wind-driven vertical mixing, a region where they no longer multiply and where cell respiration is less prevalent than photosynthesis. Furthermore, light penetration through water is highly limiting in high latitudinal regions such as British Columbia, and further hindered by onshore winds that produce abundant rainclouds that cover most of this region. Limited light occurs over relatively long temporal periods and particularly in winter months. These factors result in primary production being largely more light-limited than nutrient-limited within coastal Pacific Northwest waters (Horner *et al*. 1997).

Hobson *et al*. (1994) used stable nitrogen and carbon isotopes to reconstruct a naturally occurring marine food web for seabirds and prey on the west coast of

Vancouver Island, British Columbia. The study determined that particulate organic matter (POM) was least enriched in both stable carbon and nitrogen isotope signatures. Despite no consistent pattern of trophic enrichment, fractionation of both carbon and nitrogen stable isotopes was evident in species represented from lower (kelp, euphausiids, filter feeders) to higher trophic levels (planktivorous fish, squid, seabirds). These results are in accordance with other studies that determine higher latitude primary producers to be ^{13}C-depleted in relation to lower latitudes (Rau *et al*. 1982; Goericke & Fry 1994; Schell *et al*. 1998).

Essential to measuring isotopic changes to food webs over varying temporal scales is the awareness that different tissues within an individual under study exhibit different isotopic values and there are often varying rates of tissue turnover. This turnover is attributable to growth and metabolic tissue replacement (Perga & Gerdeau 2005). 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 (Tieszen *et al*. 1983; Hobson & Clark 1992). Unless the study organism is an indiscriminant feeder, selecting tissues with higher turnover rates for isotopic analysis are ideal when performing seasonal comparative studies (Simenstad & Wissmar 1985; Goering *et al*. 1990; Riera & Richard 1997; Buskey *et al*. 1999; Kang *et al*. 1999). Often the diets of consumers vary over time, and this is not immediately manifested in its isotopic signature (Sweeting *et al*. 2005). There is often a lag time. Vizzini & Mazzola (2003) analyzed seasonal variation in isotopic compositions within a Mediterranean coastal lagoon food web and found a general depletion of values in winter and enrichment of values in summer. They sampled dorsal muscle tissue of lagoon fish species, as such this tissue was found to respond relatively quickly to dietary changes per season, and less variable than in other organs (Pinnegar & Polunin 1999). Aside from muscle, any other variety of protein fractions can also be used as indicators of diet (Peterson & Fry 1987).

Monitoring seasonal variation of isotopic concentrations within tissues of IMTA-reared extractive organisms would be advantageous to ecological and economical management. This knowledge can be applied towards predicting carrying and production capacity that would permit optimal growth, feed conversions, fish health, and reduce instance of disease and mortality while maximizing economic returns (Losordo & Westers 1994). Many aquaculture systems have been investigated with respect to carrying capacities resulting in mathematical models; many of which assume species to be feeding within the same feeding guilds and thus competing for the same resources (Prins *et al*. 1998). Competition for niche space in natural communities is commonly understood (Connell 1961), and inter- and intraspecific competition for resources and space by adjacent, unintended epibiont communities should be factored into carrying capacity studies within IMTA facilities. Studies have demonstrated the potential for fouling communities, growing adjacent to shellfish monocultures, to develop and possibly compete with these intended reared organisms (Lesser *et al*. 1992; Mazouni *et al*. 2001; Dubois *et al*. 2007). To date, no studies have monitored the presence or absence of competition for food and space between intended extractive organisms within IMTA facilities and adjacent epibiont fouling communities over space and time.

1.3 Thesis objectives

The influence of aquaculturally derived organic waste on intended extractive and biofouling organisms have received scant investigation, particularly through stable isotope analysis. Furthermore, no studies have attempted to monitor this influence spatially and temporally. With this thesis, I would like to attempt to answer some essential questions pertaining to how the presence of an IMTA facility affects the isotopic signatures of both intended and naturally occurring marine biological communities adjacent to the facility. The goals of this research were to:

(1) Evaluate the effects of IMTA-derived organic effluent on the isotopic signatures of intended extractives and unintended adjacent biofouling communities;

(2) Measure the degree of inter- and/or intraspecific variability among and within both intended extractive and unintended biofouling organisms;
(3) Identify temporal variability of resource partitioning among intended and unintended organisms.

In order to meet these objectives, I measured the $\delta^{13}C$ and $\delta^{15}N$ stable isotope compositions of intended extractive and biofouling organisms found within and adjacent to an IMTA facility located in Kyuquot Sound, British Columbia each month for approximately one year. In Chapter 2, I compare carbon and nitrogen stable isotope signatures of both IMTA site and reference site blue mussels *Mytilus edulis* and biofouling brooding transparent tunicates *Corella inflata* in order to investigate degree of resource partitioning and inter- and intraspecific competition. In Chapter 3, I measure any temporal variation of trophic linkages among intended extractive organisms and unintended biofouling communities within and adjacent to an IMTA food web reconstructed using carbon and nitrogen stable isotope signatures. I conclude in Chapter 4 with a summary of the research presented in this thesis, and discuss the possible implications of IMTA-derived organic effluent subsidies on the surrounding marine ecosystem.

Literature Cited

Barchach JE (1986). Constraints in polyculture. *Aquacult Eng* 5: 287—300.

Brooks KM, Mahnken CVW (2003). Interactions of Atlantic salmon in the Pacific northwest environment: II. Organic wastes. *Fish Res* 62: 255—293.

Buskey EJ, Dunton KH, Parker PL (1999). Variations in stable isotope of the copepod *Acartia tonsa* during the onset of the Texas brown tide. *Estuaries* 22: 1018—1026.

Chopin T, Buschmann AH, Halling C, Troell M, Kautsky N, Neori A, Kraemer GP, Zertuche-Gonzalez JA, Yarish C, Neefus C (2001). Integrating seaweeds into marine aquaculture systems: a key towards sustainability. *J Phycol* 37: 975—986.

Coen I, Neori A (1991). *Ulva lactuca* biofilters for marine fishpond effluents. I. Ammonia uptake kinetics and nitrogen content. *Bot Mar* 34: 475—482.

Connell JH (1961). The influence of interspecific competition and other factors on natural populations of barnacle *Balanus balanoides*. *Ecol Monogr* 31: 61—104.

Conover RJ, Francis V (1973). The use of radioactive isotopes to measure the transfer of materials in aquatic food chains. *Mar Biol* 18: 272—283.

DeNiro MJ, Epstein S (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 42: 495—506.

Dubois S, Orvain F, Marin-Léal JC, Ropert M, Lefebvre S (2007). Small-scale spatial variability of food partitioning between cultivated oysters and associated suspension-feeding species, as revealed by stable isotopes. *Mar Ecol Prog Ser* 336: 151—160.

Duggins DO (1980). Kelp beds and sea otters: an experimental approach. *Ecology* 61: 447—453.

Dunne JA, Williams RJ, Martinez ND (2004). Network structure and robustness of marine food webs. *Mar Ecol Prog Ser* 273: 291—302.

Elton C (1927). *Animal Ecology*. Sedgwick & Jackson Ltd., London.

Ervik A, Hansen PK, Aure J, Stigebrandt A, Johannessen P, Jahnsen T (1997). Regulating the local environmental impact of intensive marine fish-farming I. The concept of the MOM system (Modelling-Ongrowing fish farms-Monitoring). *Aquaculture* 158: 85—94.

Estes JA, Palmisano JF (1974). Sea otters: their role in structuring nearshore communities. *Science* 185: 1058—1060.

Folke C, Kautsky N (1989). The role of ecosystems for a sustainable development of aquaculture. *Ambio* 18: 234—243.

Folke C, Kautsky N (1991). Aquaculture with its environment: prospects for sustainability. *Ocean Coast Mgmt* 17: 5—24.

Frederiksen S (2003). Food web studies in a Norwegian kelp forest based on stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis. *Mar Ecol Prog Ser* 260: 71—81.

Gao Q-F, Shin PKS, Lin G-H, Chen S-P, Cheung SG (2006). Stable isotope and fatty acid evidence for uptake of organic waste by green-lipped mussels *Perna viridis* in a polyculture fish farm system. *Mar Ecol Prog Ser* 317: 273—283.

Goericke R, Fry B (1994). Variations of marine $\delta^{13}C$ with latitude, temperature and dissolved CO_2 in the world ocean. *Global Biochem Cycles* 8: 85—90.

Goering J, Alexander V, Haubenstock N (1990). Seasonal variability of stable carbon and nitrogen isotope ratios of organisms in the North Pacific bay. *Estuar Coast Shelf Sci* 30: 239—260.

Gowen RJ, Bradbury NB (1987). The ecological impact of salmonid farming in coastal waters: a review. *Oceanogr Mar Biol Ann Rev* 25: 563—575.

Hairston NG, Smith FE, Slobodkin LB (1960). Community structure, population control, and competition. *Am Nat* 94: 421—425.

Hobson KA, Clark RG (1992). Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. *Condor* 94: 179—186.

Hobson KA, Piatt JF, Pitocchelli J (1994). Using stable isotopes to determine seabird trophic relationships. *J Anim Ecol* 63: 786—798.

Horner RA, Garrison DL, Plumley FG (1997). Harmful algal blooms and red tide problems on the US West Coast. *Limnol & Oceanogr* 42: 1076—1088.

Kang CK, Sauriau PG, Richard P, Blanchard GF (1999). Food sources of the infaunal suspension-feeding bivalve *Cerastoderma edule* in a muddy sandflat of Marennes-Oleron Bay, as determined by analyses of carbon and nitrogen stable isotopes. *Mar Ecol Prog Ser* 187: 147—158.

Karakassis I, Tsapakis M, Hatziyanni E, Papadopoulou KN, Plaiti W (2000). Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. *ICES J Mar Sci* 57: 1462—1471.

Lesser MP, Shumway SE, Cucci T, Smith J (1992). Impact of fouling organisms on mussel rope culture: interspecific competition for food among suspension-feeding invertebrates. *J Exp Mar Biol Ecol* 165: 91—102.

Levings CD (1997). Waste discharge. In: *Salmon Aquaculture Review*. Environmental Assessment Office, Stn Prov Govt Vic, BC, Canada pp. WD 1—47.

Lin CK (1989). Prawn culture in Taiwan, what went wrong? *World Aquacultur*e 20: 19—20.

Link J (2002). Does food web theory work for marine ecosystems? *Mar Ecol Prog Ser* 230: 1—9.

Losordo TM, Westers H (1994). System carrying capacity and flow estimation. In: Timmons MB, Losordo TM (eds) (1994). *Aquaculture Water Reuse Systems: Engineering Design and Management* Elsevier pp. 9—60.

Marion A, Harvey M, Chabot D, Brêthes JC (2008). Feeding ecology and predation impact of the recently established amphipod, *Themisto libellula*, in the St. Lawrence marine system, Canada. *Mar Ecol Prog Ser* 373: 53—70.

Mazouni N, Gaertner J-C, Deslous-Paoli J-M (2001). Composition of biofouling communities on suspended oyster cultures: an in situ study of their interactions with the water column. *Mar Ecol Prog Ser* 214: 93—102.

Mazzola A, Mirto S, Danovaro R (1999). Initial fish-farm impact on meiofaunal assemblages in coastal sediments of the western Mediterranean. *Mar Poll Bull* 38: 1126—1133.

Mazzola A, Sarà G (2001). The effect of fish farming organic waste on food availability for bivalve molluscs (Gaeta Gulf, Central Tyrrhenian, MED): stable carbon isotopic analysis. *Aquaculture* 192: 361—379.

McGhie TK, Crawford CM, Mitchell IM, O'Brien D (2000). The degradation of fish-cage waste in sediments during fallowing. *Aquaculture* 187: 351—366.

Michener RH, Schell DM (1994). Stable isotope ratios as tracers in marine aquatic food webs. In: *Stable Isotopes in Ecology and Environmental Science*. Blackwell Scientific Publications, pp. 138—157.

Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MCM, Clay J, Folke C, Lubchenko J, Mooney H, Troell M (2000). Effect of aquaculture on world fish supplies. *Nature* 405: 1017—1024.

Neori A, Chopin T, Troell M, Buschmann AH, Kraemer GP, Halling C, Shpigel M, Yarish C (2004). Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture* 231: 361—391.

Oksanen L, Fretwell SD, Arruda J, Niemela P (1981). Exploitation ecosystems in gradients of primary productivity. *Am Nat* 118: 240—261.

Paine RT (1980). Food webs: linkage, interaction strength and community infrastructure. *Anim Ecol* 49: 666—685.

Paine RT (1988). Food webs: road maps of interactions or grist for theoretical development? *Ecology* 69: 1648—1654.

Pandian TJ (1976). Mechanisms of heterotrophy. In: O. Kinne (ed.), *Marine Biology*, Vol II. John Wiley, New York, NY, pp. 179—201.

Parsons TR, Takahashi M, Hargrave B (1977). *Biological Oceanographic Processes*, Pergamon Press, New York, 332p.

Perga ME, Gerdeaux D (2005). 'Are fish what they eat' all year round? *Oecologica* 144: 598—606.

Peterson BJ, Fry B (1987). Stable isotopes in ecosystem studies. *Ann Rev Ecol Syst* 18: 293—320.

Pimm SL (1982). *Food webs*. Chapman & Hall, New York.

Pinegar JK, Polunin NVC (1999). Differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. *Funct Ecol* 225—231.

Polis GA (1991). Complex trophic interactions in deserts: an empirical critique of food-web theory. *Am Nat* 138: 123—155.

Polis GA, Strong DR (1996). Food web complexity and community dynamics. *Am Nat* 147: 813—846.

Polis GA, Anderson WB, Holt RD (1997). Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Ann Rev Ecol Syst* 28: 289—316.

Primavera JH (1991). Intensive prawn farming in the Philippines: ecological, social and economic implications. *Ambio* 20: 28—33.

Primavera JH (2006). Overcoming the impacts of aquaculture on the coastal zone. *Ocean Coast Mgmt* 49: 531—545.

Prins TC, Smaal AC, Dame RF (1998). A review of feedbacks between bivalve grazing and ecosystem processes. *Aquat Ecol* 31: 349—359.

Rau GH, Sweeney RE, Kaplan IR (1982). Plankton ^{13}C:^{12}C ratio changes with latitude: differences between northern and southern oceans. *Deep-Sea Res* 29: 1035—1039.

Reid GK, Liutkus M, Robinson SMC, Chopin T, Blair T, Lander T, Mullen J, Page F, Moccia RD (2008). A review of the biophysical properties of salmonid faeces: implications for aquaculture waste dispersal models and integrated multi-trophic aquaculture. *Aquacul Res* 40: 257—273.

Reid GK, Robinson SMC, Chopin T, Mullen J, Lander T, Sawhney M, Macdonald BA, Haya K, Burridge L, Page F, Ridler N, Boyne-Travis S, Sewester J, Marvin R, Szmerda M, Powell F (2008). Recent developments and challenges for open-water, Integrated Multi-Trophic Aquaculture (IMTA) in the Bay of Fundy, Canada. *Bull Aquacul Assn Can*. Special pub 12. In press.

Riera P, Richard P (1997). Temporal variation of $\delta^{13}C$ in particulate organic matter and oyster *Crassostrea gigas* in Marennes-Oleron Bay (France): effect of freshwater inflow. *Mar Ecol Prog Ser* 147: 105—115.

Ruis JM, Marco-Méndez C, Sánchez-Lizaso JL (2010). Remote influence of offshore fish farm waste on Mediterranean seagrass (*Posidonia oceana*) meadows. *Mar Environ Res* 69: 118—126.

Sarà G, Scilipoti D, Mazzola A, Modica A (2004). Effects of fish farming waste to sedimentary and particulate organic matter in a southern Mediterranean area (Gulf of Castellammare, Sicily): a multiple stable isotope study ($\delta^{13}C$ and $\delta^{15}N$). *Aquaculture* 234: 199—213.

Sarà G, Zenone A, Tomasello A (2009). Growth of *Mytilus galloprovincialis* (mollusca, bivalvia) close to fish farms: a case of multi-trophic aquaculture within the Tyrrhenian Sea. *Hydrobiologia* 636: 129—136.

Schell DM, Barnett BA, Vinette KA (1998). Carbon and nitrogen isotope ratios in zooplankton of the Bering, Chukchi, and Beaufort Seas. *Mar Ecol Prog Ser* 162: 11—23.

Schindler DE & Lubetkin SC (2004). Using stable isotopes to quantify material transport in food webs. In: *Food Webs at the Landscape Level* (eds. Polis GA, Power ME, Huxel GR). University of Chicago Press, pp. 25—42.

Silvert W (1994). Modeling benthic deposition and impacts of organic matter loading. In: Hargreaves BT (ed.) Modeling benthic impacts of organic enrichment from marine aquaculture. *Can Tech Rep Fish Aquat Sci* 1949: 1—30.

Simenstad CA, Wissmar RC (1985). $\delta^{13}C$ evidence of the origins and fates of organic carbon in estuarine and nearshore food webs. *Mar Ecol Prog Ser* 22: 141—152.

Smith DF, Bulleid NC, Campbell R, Higgins HW, Rowe F, Tranter DJ, Tranter H (1979). Marine food-web analysis: An experimental study of demersal zooplankton using isotopically labeled pre species. *J Mar Biol* 54: 49—59.

Sweeting CJ, Jennings S, Polunin NVC (2005). Variance in isotopic signatures as a descriptor of tissue turnover and degree of omnivory. *Funct Ecol* 19: 777—784.

Su H-M, Liao IC, Chiang Y-M (1993). Mass mortality of prawn caused by *Alexandrium tamarense* blooming in a culture pond in southern Taiwan. In: Smayda TJ, Shimizu Y (eds). *Toxic phytoplankton blooms in the sea*, Elsevier, Amsterdam pp. 329—333.

Tieszen LL, Bouton TW, Tesdahl KG, Slade NA (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. *Oecologia* 57: 32—37.

Troell M, Berg H (1997). Cage fish farming in the tropical lake Kariba: impact and biogeochemical changes in sediment. *Aquacult Res* 28: 527—544.

Vizzini S, Mazzola A (2003). Seasonal variations in the stable carbon and nitrogen isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of primary producers and consumers in a western Mediterranean coastal lagoon. *Mar Biol* 142: 1009—1018.

Wang WX, Fisher NS (1999). Assimilation efficiencies of chemical contaminants in aquatic invertebrates: A synthesis. *Environ Tox Chem* 18: 2034—2045.

2.0 VARIABILITY IN FOOD SOURCE PARTITIONING AMONG BIOFOULING SUSPENSION FEEDING SPECIES WITHIN AN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEM BASED ON $\delta^{13}C$ AND $\delta^{15}N$ ISOTOPES ANALYSIS

2.1 Abstract

Biofouling epibionts are common to aquaculture facilities, and may compete for organic effluent subsidies intended for extractives harvested in an integrated multi-trophic aquaculture (IMTA) facility. To evaluate the relative contribution of aquaculturally-derived effluent to the diets of biofouling organisms, carbon and nitrogen isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of blue mussels *Mytilus edulis* and brooding transparent tunicates *Corella inflata* were sampled from the IMTA facility within Kyuquot Sound, British Columbia during winter (October 2009 and March 2010). Results were compared to those sampled from a reference site approximately 500 m away. A 3-source mixing model was employed to quantify intra- and interspecific competition between *M. edulis* and *C. inflata* in order to make inferences on the feasibility of rearing *M. edulis* alongside other epibiont species. IMTA-sampled *M. edulis* had the smallest amount of intraspecific variation and mean $\delta^{13}C$ and $\delta^{15}N$ signatures showed the least overlap with other samples. By March 2010, $\delta^{13}C$ signatures of all samples studied became less enriched—possibly owing to a general increasing production of marine phytoplankton. Both *M. edulis* and *C. inflata* showed opposing trends in $\delta^{15}N$ signatures over time, possibly indicative of an apparent lack of competition for food resources between the two epibiont species. Mixing model results confirmed this, indicating fish feed the most important food source for *M. edulis*, and the least important food source for IMTA site sampled *C. inflata*. These results confirm the utility of employing stable isotopes analysis qualitatively and quantitatively to enhance understanding of trophic linkages and resource partitioning within an integrated multi-trophic aquaculture facility.

2.2 Introduction

Commercial-scale marine fish farms release to the surrounding water column significant amounts of particulate organic and inorganic matter largely in the form of detritus—comprised mainly of uneaten fish feed and fish faecal material (Ye *et al*. 1991; Wu *et al*. 1994; Wu 1995; Mazzola & Sarà 2001; Yokoyama *et al*. 2002; Kutti *et al*. 2007). Large-scale fish farms tend to place large numbers of fish into small, confined spaces—thereby giving rise to such impacts as disease, infection, and highly concentrated effluent waste (Wu 1995). The resulting organic and inorganic effluent disperses and enriches the surrounding water column, thus affecting biotic and abiotic processes and creating changes to the abundance and diversity of nearby infaunal communities (Pearson & Rosenberg 1978; Weston 1990; Hargrave 1993; Wu 1995; Karakasis *et al*. 1999; Naylor *et al*. 2000). Many studies have researched the possibility that this particulate waste could serve as a food source to other filter feeding and detrital species which, in turn, could potentially serve to reduce these negative aquaculturally related environmental impacts (Shpigel & Baylock 1991; Shpigel *et al*. 1991, 1993b, 1997; Mazzola & Sarà 2001; Yokoyama *et al*. 2002; Gao *et al*. 2006; Dubois *et al*. 2007; Reid *et al*. 2010). Furthermore, this food source for one or more commercially marketable organisms could provide additional economic benefits and profitability for commercial aquaculture projects (Shpigel *et al*. 1993b; Troell *et al*. 2003). Hence, these benefits provide the impetus behind the expansion of polycultures and integrated multi-trophic aquaculture (IMTA) projects (Yi & Fitzsimmons 2004; Bunting 2008; Martínez-Porchas *et al*. 2010).

IMTA systems operate similar to naturally occurring food webs, with fluctuating nutrient and detrital subsidies connecting consumers and producers within and around adjacent habitats (Polis *et al*. 1997). Apart from polycultures, which are systems that simply co-culture various adjacent species, the species within the IMTA system occupy different trophic levels within a food chain (Neori *et al*. 2004, 2007; Chopin 2006). As with any naturally occurring marine ecosystem, the organisms within the IMTA require a particular set of abiotic and biotic interactions with which to survive. Faecal waste from

upper trophic level organisms, such as fish and shrimp, become food for other intermediate trophic level organisms placed within the system and, as is the case for marine aquaculture systems, require water transfer through a particular combination of gravity and currents. Typically, intermediate trophic level organisms include filter feeders, such as bivalves, and bottom feeders, such as sea cucumbers and sea urchins. Various species of harvestable seaweeds are often placed within IMTA systems and use soluble ammonia and phosphate also subsidized by the excretory effluent of the upper trophic organisms (Neori *et al*. 2004).

Increasingly, research has been conducted on fish farm waste as a potential food source for intended secondary and tertiary species, also known as extractives, within polyculture farms and IMTA projects (mussels, *Mytilus galloprovincialis*, clams, *Tapes* sp., with seabass, *Dicentrarchus labrax*, and seabream, *Sparus aurata*, Mazzola & Sarà 2001, Sarà *et al*. 2009; green-lipped mussels, *Perna viridis*, and shrimp, *Fenneropenaeus merguiensis* and *Penaeus monodon*, Yokoyama *et al*. 2002; green-lipped mussels, with groupers, *Epinephelus awoara*, snappers, *Lutjanus russellii*, and seabream, *Acanthopagrus talus*, Gao *et al*. 2006; blue mussels, *Mytilus edulis*, and Atlantic salmon, *Salmo salar*, Reid *et al*. 2010; chlorophyte seaweed, *Ulva* sp., with red sea bream, *Pagrus major*, and yellowtail, *Seriola quinqueradiata*, Yokoyama & Ishihi 2010). There is, however, a dearth of studies on potential competition for food between farmed species and adjacent wild sessile epibionts that are considered biofoul, or in this case, species unintended for harvest that recruit to rearing aquaculture nets and other associated structures (Lesser *et al*. 1992; Dubois *et al*. 2007). It is suspected that organisms living and feeding within the same trophic niche space would also compete for the same available nutritive resources (Prins *et al*. 1998). Dubois *et al*. (2007) analyzed the spatial variability of, as well as the intra- and interspecific food partitioning between, farm-reared oysters and classic fouling epibionts such as barnacles, serpulids, terebellid polychaetes, and ascidians. They found that farmed oysters, *Crassostrea gigas*, do not necessarily compete for food with these co-occurring epibionts. Naturally occurring particulate organic matter (POM) and microphytobenthos (MPB) may also provide a food source, and not just aquaculturally generated waste alone. Biotic interactions with

adjacent, non-aquaculture species are worth exploring because they may pose a competitive threat to intended extractive species, which may consequently affect the ability to predict the carrying capacity of aquaculturally harvestable extractives and, consequently, the economic success of the facility. This study attempts to do so.

Two of the most common, early recruiting epibiont species were sampled and analyzed for intraspecific and interspecific competition with intended aquaculture-reared organisms. Blue mussel *Mytilus edulis* and the brooding transparent tunicate *Corella inflata* are two species of soft-bodied epibionts that typically biofoul to aquacultural structures within the temperate coastal waters of the Pacific Northwest. They are both considered solitary organisms that form communities that follow Connell and Slatyer's (1977) inhibition model of succession (Greene *et al*. 1983). When there is primary space available, solitary organisms gradually increase in abundance until there is no more space to occupy. In line with the inhibition model, these species typically inhibit the invasion of new recruits, suppress the growth of co-habitating resident species, and have a high propensity for self replacement. It is difficult to ascertain whether competition for food sources is occurring within shellfish nets of fish farms, polycultures and IMTA systems, given the range of suspension feeding biofouling colonies that they support.

Non native *M. edulis* occur both intertidally and subtidally along the Pacific Northwest coast (Lamb & Hanby, 2005). In terms of feeding behaviour, mussels are indiscriminant or generalist consumers and can filter particles greater than 2 to 5 µm with 100% efficiency (Bayne *et al*. 1977; Dame 1996); however, depending on the size of the organism, can reach a plateau of suspended particle size where the species' ability to filter slows to a rate of zero (Widdows *et al.* 1979). Mussels also produce pseudofaeces, which is material that has been filtered but rejected by the gills and palps (Widdows *et al*. 1979). Rejection of particles by mussels has been known to occur when particles are too large. Mussels have the ability to efficiently take up uneaten fish feed, as well as flour, in fish ponds (Yokoyama *et al*. 2002). Gao *et al*. (2006) compared carbon and nitrogen stable isotopic signatures between farm-reared green-lipped mussels and non-farmed mussels (*Perna viridis*) adjacent to farmed fish and found evidence of uptake and assimilation of fish feed and fish faeces. Other studies have shown that *M. edulis* can

serve to increase production of fish farms by recycling excess aquaculturally-derived algal biomass (Larsson 1985; Jones & Iwama 1991). In Scotland Stirling and Okumus (1995) found that blue mussels grown within salmon farms showed increased body growth compared to those grown within shellfish farms. This is probably due to the increased level of particulate organic matter and temperature found within fish farms. Furthermore, growth was variable with season; mussels demonstrated increased growth during summer months as opposed to negative growth and production in winter months. By placing them within an IMTA system downstream from fish cages, mussels can potentially act to regulate cycling of organic particulate waste generated by farmed fish, thereby creating potential financial benefits in addition to a reduction in pollution (Folke & Kautsky 1989; Shpigel *et al*. 1997).

Like *M. edulis*, the brooding transparent tunicate *Corella inflata* are commonly found as members of biofouling communities; often collected on floats, pilings, and aquaculture nets and pens (Lambert *et al.* 1981). *C. inflata* is a solitary, self-fertilizing phlebobranch ascidian, geographically spread throughout coastal Washington and British Columbia, and confined to shallow, intertidal depths (0.1—18m). To date, there are no studies that analyze populations of biofouling *C. inflata* as potential competitors to food sources within and around IMTA systems.

Direct gut content analysis has been considered the conventional method in various studies for food source evaluation and for resolving of food web structures (Kamermans 1994; Lehane & Davenport 2002; Marion *et al*. 2008). However due to the small size of food particles within stomachs of suspension feeders like *M. edulis* and *C. inflata*, as well as to the high degree of uncertainty as to the precise identity of many microalgal species, this method may result in error. Additionally, gut content analysis only displays a snapshot of food source assimilation at the time of analysis—without considering turnover of nutrients within tissues over longer period of time.

The present study uses stable carbon and nitrogen isotopes as analytical tools to identify whether the organic waste generated by farmed sablefish *Anoplopoma fimbria* (*ie*. faecal matter and uneaten fish feed originating from an IMTA facility) is present in

the body tissues of co-occurring blue mussels *M. edulis* and brooding transparent tunicates *C. inflata*. Use of stable isotopic analysis is currently considered the most ideal method for identifying food sources within the tissues of benthic consumers such as filter feeders and epibionts (Peterson & Fry 1987). Stable isotopic carbon and nitrogen, for example, exists in trace amounts within all organic material. $\delta^{13}C$ signatures within organic tissues of consumers are slightly enriched relative to the levels that exist in their food source (DeNiro & Epstein 1978). This slight enrichment, or the difference between the isotopic signature of a consumer and its food source, is known as metabolic fractionation, and is averaged to be approximately 1—1.5 ‰ for marine invertebrates (DeNiro & Epstein 1978). $\delta^{15}N$ signatures within tissues tend to show greater predictable fractionation (approximately 3 ‰ enrichment) relative to food sources, which proves more useful for the identification of trophic position within a food web.

I hypothesize that the isotopic signatures of uneaten fish feed and faecal material from the farmed sablefish *A. fimbria* will be detected in adjacent mussels *M. edulis* and transparent brooding tunicates *C. inflata*, however at more predictably enriched signatures relative to the IMTA effluent materials. Since sampling will occur during winter months, consumer signatures are presumed to not be affected by those of particulate organic matter sources that generally measure abundantly high in summer months. Furthermore, I hypothesize that these signatures will be more significantly enriched than those detected in tissues of the same species found at the reference site located approximately 500 m from the IMTA research and development project. It is believed that this data will provide insight into the feasibility and capability of mussels *M. edulis* as potential and harvestable biofilters that can both increase profitability and reduce pollution in a large scale IMTA project.

The objectives of the present study were to (a) create a dual isotope plot ($\delta^{13}C$ and $\delta^{15}N$) in order to identify spatial and temporal variability of food partitioning between IMTA farm versus reference site *M. edulis* and *C. inflata*; to (b) use mixing models to quantify the contribution of uneaten fish feed, fish excretion, and marine particulate organic matter (from previously published data) as potential food sources for *M. edulis*

and *C. inflata* biofouled to nearby rearing IMTA nets; and to (c) establish overall trophic relationships of all measured samples within an IMTA food web.

2.3 Materials and Methods

2.3.1 Study Site

The IMTA research and development study site is situated on the northwest region of Vancouver Island, British Columbia (50°3’10”N 127°18’45”W) just off Surprise Island, Kyuquot Sound and approximately 4.5 kilometers from the village of Kyuquot. The approximately 5 km^2 marine study area is characterized by circular tidal flow, with a depth that is mostly photic, at approximately 30 m. A nearby anadromous fish stream, British Creek, discharges relatively low flows fresh water into the site. Current flow rate is low and runs laterally through a series of seven sablefish cages (50 x 50 ft^2, 60 ft deep) ranging approximately 4,500 to 10,000 fish per cage. Current continues through to a series of 250 shellfish droplines spaced one metre apart and are deployed in a raft system that is approximately 14 metres across x 75 metres long. Each dropline has 12 tiers, the top of which is approximately 5 metres deep with each tier housing approximately 25 to 50 Pacific scallops (*Patinopectin yessoensis*). The current finally 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 set up as a sampling reference site. This was accomplished by setting a series of five shellfish lantern nets for natural recruitment.

Figure 4 Map of Vancouver Island, Canada featuring study site at Surprise Island (indicated by arrow).

Figure 5 Satellite image of Surprise Island, BC at larger scale (indicated by arrow).

2.3.2 Sample Collection

Samples within the sea cages and shellfish pens were collected monthly from October 2009 to March 2010. Sablefish *Anoplopoma fimbria* samples were obtained from the individuals harvested by Pacific SEA-Lab, proprietor of the IMTA facility; blue mussels *M. edulis* and tunicates *C. inflata* samples were manually retrieved from an adjacent series of shellfish nets. Sampling was duplicated at a reference site located approximately 500 m from the site. Previous studies have demonstrated that dispersion of fish farm waste rarely exceeds 100 m (Ye *et al*. 1991; Wu *et al*. 1994). Shellfish nets at the reference site were suspended two months prior to the first sampling effort (October 2009) permitting recruitment to take place.

All samples were dissected and initially processed in a laboratory on site. No studies have shown differences in tissue turnover time among organs of both blue mussels and brooding transparent tunicates, and thus entire soft bodies were sampled (dissected from the shell and operculae) using a scalpel and surgical blade (Dubois *et al*. 2007). Collection of sablefish faeces was carried out through the removal of colon, followed by the use of pipettes for further extraction. Since $\delta^{13}C$ materials are highly enriched with inorganic carbonates that could potentially influence results in $\delta^{15}N$ signatures, therefore all tissues were soaked in 5% HCl and thoroughly rinsed in distilled water in order to remove inorganic carbonates prior to drying (Simenstad & Wissmar 1985; Bosley & Wainright 1999; Pinnegar & Polunin 1999; Kaehler & Pakhomov 2001). Dissected tissue samples, faecal matter, as well as samples of commercial fish feed (Taplow Aquafeed©) were oven-dried at 60ºC for a minimum of 24h to constant dry weight, then ground to a fine powder using a pestle and mortar (Sarà *et al*. 2004). 1 mg samples of all tissues were weighed in tin combustion containers (6 x 4 mm) in preparation for sample continuous flow isotope ratio mass spectrometry (CF-IRMS) analysis.

2.3.3 Isotopic Analysis

Isotopic measurements were performed at the University of California—Davis Stable Isotope Facility. CF-IRMS analysis was completed using a Europa Hydra 20/20 continuous-flow isotope ratio mass spectrometer. Analytical error of $\delta^{13}C$ and $\delta^{15}N$ were

determined using replicates of laboratory standards. Data results are expressed in the standard delta notation (parts per thousand (‰) deviations from a standard; Pee Dee Belemnite (PDB) for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$) as:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where X is either carbon (C) or nitrogen (N) and R is the ratio of heavy to light stable isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). Triplicate samples were analyzed haphazardly and resulted in an analytical reproducibility of 0.25 ‰ for $\delta^{13}C$ and 0.43 ‰ for $\delta^{15}N$.

2.3.4 Literature Review for $\delta^{13}C$ and $\delta^{15}N$ of Marine Particulate Organic Matter

Since on-site sample data of marine particulate organic matter (POM) is missing, I reviewed the literature on POM relative to the diet of mussel and tunicate sample group sampled within the IMTA research and development site. This provided 4 measurements of $\delta^{13}C$ and 3 measurements of $\delta^{15}N$ for POM within similar geographic regions to Kyuquot Sound, BC. See Table 2.4 for results of literature review.

2.3.5 Three-end Member Isotope Mixing Model

Many studies use isotopic mixing models to evaluate the proportion of each food source to the diet of various consumers in marine systems (Bustamante *et al*. 1996; Kaehler *et al*. 2000; Gao *et al*. 2006; Nadon & Himmelman 2006). The present study recognizes fish feed, fish excretion and marine POM as potential food sources for marine sessile epibionts *M. edulis* and *C. inflata*. As such, I sought to determine the percent contribution of these IMTA-derived primary producers along with POM values within consumers using the following 3-source system isotope mixing model described by Phillips (2001):

$$(\delta^{13}C'_A - \delta^{13}C_D)[C]_A f_{A,BM} + (\delta^{13}C'_B - \delta^{13}C_D)[C]_B f_{B,BM} + (\delta^{13}C'_C - \delta^{13}C_D)[C]_C f_{C,BM} = 0$$

$$(\delta^{15}N'_A - \delta^{15}N_D)[N]_A f_{A,BM} + (\delta^{15}N'_B - \delta^{15}N_D)[N]_B f_{B,BM} + (\delta^{15}N'_C - \delta^{15}N_D)[N]_C f_{C,BM} = 0$$

$$f_{A,BM} + f_{B,BM} + f_{C,BM} = 1$$

where $f_{A,B}$, $f_{B,B}$, and $f_{C,B}$ represent the fractional contributions of assimilated biomass (*BM*) of the potential food sources *A*, *B*, and *C*, within the mixture *D*. Carbon concentrations in the food sources are represented in the model by $[C]_A$, $[C]_B$, $[C]_C$; nitrogen concentrations are represented as $[N]_A$, $[N]_B$, $[N]_C$. As noted earlier, $\delta^{13}C_{POM}$ was determined by averaging the values obtained from previously published studies. Trophic fractionation is represented by the prime (') symbol within the above model. For studies dealing with proportion of food source contributions, step-wise enrichment of carbon and nitrogen stable isotopes (*ie*. fractionation) requires consideration (Dubois *et al*. 2007). By measuring fractionation of $\delta^{13}C$, basal sources of productivity and primary producers can be defined (DeNiro & Epstein 1978; Fry & Sherr 1984). Measuring fractionation of $\delta^{15}N$ can describe trophic level of species under examination (Gannes *et al.* 1997; Sweeting *et al.* 2005). Generally, fractionation values of each species increases consistently and predictably per trophic level, relative to the signatures of their food source. Many studies have determined that the average ^{13}C enrichment is equal to 1 ‰ and the average ^{15}N enrichment is equal to 3 ‰ per trophic level (Peterson & Fry 1987; Deegan & Garrett 1997; Kharlamenko *et al.* 2001; Riera *et al.* 2002). Therefore, these values were used for the three-source isotopic mixing model. Carbon and nitrogen concentration and contribution to consumer (*M. edulis* and *C. inflata*) diets were calculated with the use of the software IsoSource version 1. 3. 1 (Phillips & Gregg 2003), which calculates and compiles all possible feasible solutions that could explain isotopic signatures in *M. edulis* and *C. inflata*. IsoSource software sums 0 to 100 % possible combinations of potential contributions (*ie.* feasible ranges) of each food source in minimal user-specified increments (1 % in this study). Then predicted isotope values of each source mixture were calculated via linear mixing model equations that preserve mass balance (Phillips 2001; Benstead *et al*. 2006; Dubois *et al*. 2007). These values are then compared to observed isotopic values and all feasible combinations are matched and averaged within user-specified tolerance levels set to 3.3 ‰ for mussels, and 1.8 ‰ for tunicates to represent mean source proportions (Phillips & Gregg 2003; Benstead *et al*. 2006; Dubois *et al*. 2007; Quan *et al*. 2007). Trophic fractionation was accounted for within the source data prior to inputting to software for analysis. Since POM was not

measured at the site, the literature averaged values included in this model are purely hypothetical and better represent non-aquaculture effluent material.

2.3.6 Statistical Analysis

Prior to statistical analysis, all isotopic signature data were tested for normality using Kolmogorov-Smirnov test, and homogeneity of variance using Levene's test. A series of paired *t*-tests were used to determine differences between average $\delta^{13}C$ and $\delta^{15}N$ obtained within tissues of organisms found at the IMTA project and reference sites. In each test, 95% significance level was adhered to. A one-way ANOVA, followed by Tukey's HSD post hoc test, were performed to quantitatively estimate any interspecific and intraspecific differences within and among each suspension feeding group at each site (IMTA farm- and reference site mussels and tunicates, respectively), as well as any temporal variation between sampling dates for IMTA samples (Zar 1999). All statistical analyses were performed using software SPSS version 17.0 for Windows (SPSS 17.0, Chicago, IL).

2.4 Results

2.4.1 Inter- and Intraspecific Isotopic Variation in IMTA versus Reference Sites in Suspension Feeders

Figure 2.3 demonstrates that average isotopic signatures between sites varied, with both species measuring higher interspecific variation than intraspecific variation. All sampling groups demonstrated some overlap in isotopic ranges. IMTA site mussels were isotopically heavier in $\delta^{15}N$ (approx. 9 ‰) than the other sampling groups, and characterized by the lowest range of $\delta^{13}C$. The $\delta^{13}C$ signatures of reference site mussels were isotopically heavier (approx. -20 ‰) than IMTA site mussels (approx. -18.5 ‰), but not markedly different from either farm- or reference site tunicates (average values approx. -19.8 ‰ and -19.6 ‰, respectively). Reference site mussels were heavier isotopically in $\delta^{15}N$ (approx. 8 ‰) than both farm- and reference site tunicates (average values approx. 6 ‰ and 6.2 ‰, respectively) but slightly lighter than farm site mussels (approx. approx. 9 ‰). Both farm- and reference site tunicates showed very little differences in both ^{13}C and ^{15}N isotopic signatures; however, reference site ascidians had

a smaller range of $\delta^{13}C$ values (-18.49 to -20.55 ‰). IMTA farm site tunicates displayed the widest range of isotopic values overall (-9.43 to -22.26 ‰).

The one-way ANOVA of $\delta^{13}C$ signatures performed between the four sampling groups showed that there is a statistically significant difference between mean signatures (df = 3, F = 4.799, MS = 6.746, p = 0.006). A greater significant difference was observed for $\delta^{15}N$ signatures between all sampling groups (df = 3, F = 18.112, MS = 23.021, p = 0.00). Student *t*-tests using mean isotopic values between co-occurring IMTA farm site mussels and farm site tunicates revealed that both had significantly different ^{13}C (df = 23, *t* = 2.501) and ^{15}N isotopic values (df = 23, *t* = 6.419). As for co-occurring mussels and ascidians from the reference site, *t*-tests revealed the means for ^{13}C were not significantly different from each other (df = 19, *t* = -0.742) but significantly different in ^{15}N values (df = 19, *t* = 3.399).

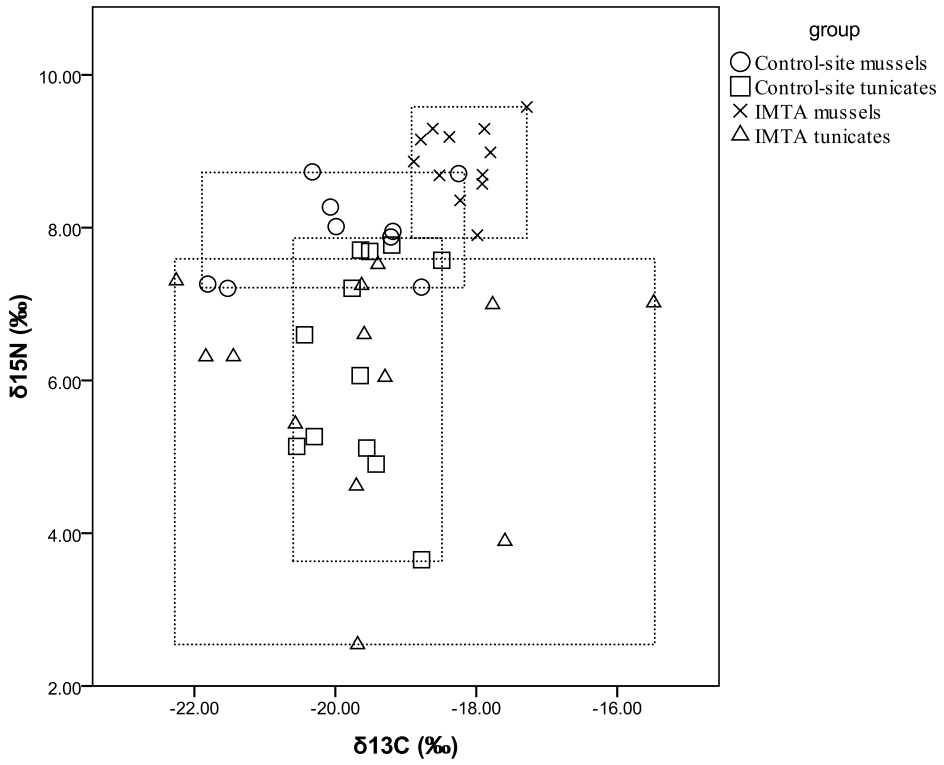


Figure 2.3 Spatial variability of average stable ^{13}carbon- and ^{14}nitrogen isotope signatures of reference site mussels, *M. edulis* (○), reference site tunicates, *C. inflata* (□), IMTA

farm vicinity mussels, *M. edulis* (x), and IMTA farm vicinity tunicates, *C. inflata* (∆). Values represent spatial variability of co-occurring filter feeding organisms by sampling site. Dashed boxes represent maximum and minimum $\delta^{13}C$ and $\delta^{15}N$ signatures within each site.

2.4.2 Consumers: Mussels Mytilus edulis *and Tunicates* Corella inflata

Tables 2.1 and 2.3 show the average values for $\delta^{13}C$ and $\delta^{15}N$ by site (IMTA versus reference) and by sampling date for blue mussels. The $\delta^{13}C$ averages over all the sampling dates were calculated as -18.18 ± 0.470 ‰ (*n* = 12) for the farm site mussels and -19.90 ± 1.198 ‰ (*n* = 9) for the reference site mussels. All sample date $\delta^{15}N$ averages were calculated at 8.88 ± 0.468 ‰ (*n* = 12) for farm colonies and at 7.92 ± 0.596 ‰ (*n* = 9) for reference site recruits. Paired Student *t*-tests were employed to determine if the means of both $\delta^{13}C$ and $\delta^{15}N$ differed between the reference and the farm sites. There was significant enrichment found between sites for $\delta^{13}C$ values (df = 19, F = 6.771, p = 4.561) and for $\delta^{15}N$ values (df = 19, F = 0.552, p = 4.163).

Table 2.1 *Mytilus edulis*. Variation in the average $\delta^{13}C$ and $\delta^{15}N$ (‰) values by site (IMTA versus reference) and by sampling date. Obs(*n)* refers to the total number of observations/specimens sampled. Values within columns with a superscripts asterix (*) are statistically similar.

Site	Sampling Date	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Obs (*n*)
Farm	Nov 22, 09	-17.98	8.64	3
	Jan 24, 10	-18.14	8.43*	3
	Feb 28, 10	-17.85*	9.35*	3
	Mar 28, 10	-18.76*	9.10	3
Reference	Nov 22, 09	-18.88	8.18	3
	Feb 28, 10	-20.71	7.23	3
	Mar 28, 10	-20.13	8.34	3

Tables 2.2 and 2.3 summarize the average values for $\delta^{13}C$ and $\delta^{15}N$ by site (farm versus reference) and by sampling date for brooding transparent tunicates *C. inflata*. The $\delta^{13}C$ average, for all dates, was calculated to be -19.76 ± 1.846 ‰ (n = 13) for farm-vicinity individuals and -19.61 ± 0.620 ‰ (n = 12) for reference site recruits. Sample date $\delta^{15}N$ mean totals were averaged to be 5.98 ± 1.493 ‰ (n = 13) for farm site individuals and 6.22 ± 1.394 ‰ (n = 12) for reference site recruits. Paired *t*-tests indicated no statistically significant difference found among $\delta^{13}C$ values (df = 23, F = 4.452, p = 0.930) and $\delta^{15}N$ values (df = 23, F = 0.029, p = 0.687) between each site over all sampling dates.

Table 2.2 *Corella inflata*. Variation in the average $\delta^{13}C$ and $\delta^{15}N$ (‰) values by site (Farm versus Reference) and by sampling date. Obs(*n*) refers to the total number of observations/specimens sampled.

Site	Sampling Date	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Obs (*n*)
Farm	Oct 25, 09	-18.12	6.55	3
	Nov 22, 09	-18.97	4.56	3
	Jan 24, 10	-20.13	5.02	2
	Feb 28, 10	-22.05	6.81	2
	Mar 28, 10	-19.54	6.94	3
Reference	Oct 25, 09	-19.31	5.80	3
	Nov 22, 09	-19.30	7.50	3
	Jan 24, 10	-19.98	6.54	3
	Mar 28, 10	-19.84	5.05	3

2.4.3 Potential Food Sources: Fish Feed, Fish Excretion, and Marine POM

The average ^{13}C and ^{15}N isotopic signatures for mussels, tunicates, fish feed and sablefish excretion are listed in Table 2.3. These values have been pooled to include all sampling months. Organic fish feed showed an average $\delta^{13}C$ value of -21.62 ± 0.461 ‰, which is similar to the results reported in related studies (Mazzola & Sarà 2001; Ye *et al.* 1991; Sutherland *et al*. 2001). I found an average $\delta^{15}N$ value of 10.48 ± 0.455 ‰. Of the 12 samples of sablefish faeces measured, the average $\delta^{13}C$ was -21.45 ± 1.083 ‰ and the average $\delta^{15}N$ was slightly enriched at 11.70 ± 1.056 ‰. When comparing all isotopic averages between fish feed and fish excretion, paired *t*-tests revealed $\delta^{13}C$ (df = 6, F = 0.582, p = 0.630) and $\delta^{15}N$ (df = 6, F = 0.003, p = 0.640) no significant differences.

Table 2.3 Carbon and nitrogen isotopic signatures of organic matter sources and consumers in both study areas. (Obs (*n*) = number of observations; $\delta^{13}C$ = carbon isotopic values (‰); $\delta^{15}N$ = nitrogen isotopic values (‰); ± s.e. = standard errors for the means). Data represent pooled averages of tissues sampled monthly between October 2009 and April 2010.

Sample group	**Obs (*n*)**	**$\delta^{13}C$ (‰)**	**± s.e.**	**$\delta^{15}N$ (‰)**	**± s.e.**
Fish feed	3	-21.62	0.461	10.48	0.455
Fish faeces	12	-21.45	1.083	11.70	1.056
M. edulis (IMTA)	12	-18.18	0.470	8.88	0.468
M. edulis (reference)	9	-19.90	1.198	7.92	0.596
C. inflata (IMTA)	13	-19.76	1.846	5.98	1.493
C. inflata (reference)	12	-19.61	0.620	6.22	1.394

Table 2.4 displays marine POM carbon isotope values taken from geographically similar previously published studies were pooled and averaged at -21.55 ‰ (*n* = 4). This value is within the range of average plankton values, -19 to -24 ‰ as reported by

Peterson & Fry (1987). Other studies report similar values for phytoplankton in other temperate marine waters (~ -21 ‰, Dauby 1989; Jennings *et al*. 1997). Average marine POM nitrogen isotope values were calculated to be 5.27 ‰ (n = 3), which is well within the -2 to +11 ‰ range also reported by Peterson & Fry (1987). These values, as well as those of uneaten fish feed and fish faeces, were used to calculate relative contribution of different potential food sources to the diets of epibiont consumers *M. edulis* and *C. inflata* from both sampling sites.

Table 2.4 Results of literature review of average marine particulate organic matter (POM) carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values from geographic regions similar to that of the present study site.

Geographic region of POM sample	**$\delta^{13}C$ (‰) Mean ± S.D.**	**$\delta^{15}N$ (‰) Mean ± S.D.**	**Reference**
Auk Bay, Alaska	-20.3 ± 0.9	2.7	Goering *et al*. 1990
Fritz Cove, Alaska	-20.3 ± 0.2	6.3	Goering *et al*. 1990
Broughton Archipelago, BC	-24.0 ± 1.0	n.d.	Sutherland *et al*. 2001
Barkley Sound, BC	-21.6 ± 0.2	6.8 ± 0.1	Hobson *et al*. 1994

2.4.4 Temporal Variation in ^{13}C and ^{15}N Signatures

Isotopic differences over all sampling dates for each species, as well as fish excretion and fish feed can be seen in Tables 2.1 & 2.2 and Fig. 2.4 & 2.5. For $\delta^{13}C$ means (Fig. 2.4), the most pronounced signature differences were with fish excretion (ranging from -15.33 ‰ in October 2009 to -21.45 ‰ in January 2010), followed by reference site *M. edulis* (ranging from -15.65 ‰ in January 2010 to -21.16 ‰ in March 2010), and reference site *C. inflata* (ranging from -17.56 ‰ in October 2009 to -22.82 ‰ in March 2010). Most mean $\delta^{13}C$ signatures reached their most enriched value by the March sampling date.

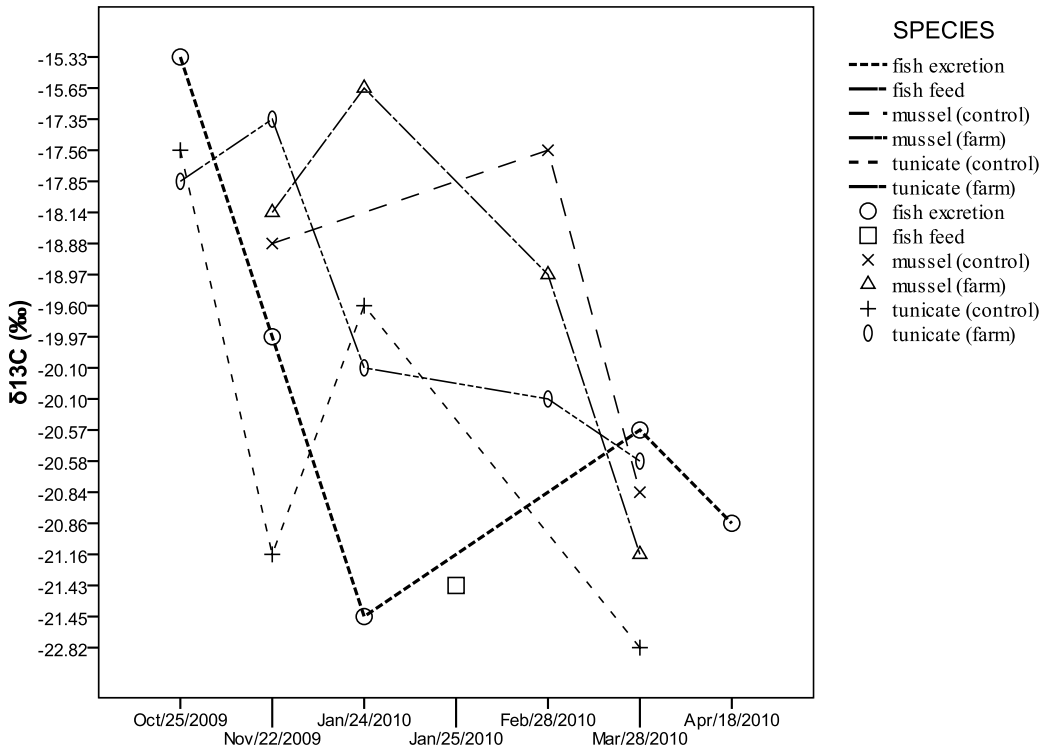


Figure 2.4 Temporal $\delta^{13}C$ variation (‰) for fish excretion (○), fish feed (□), reference site mussels *M. edulis* (x), IMTA farm site mussels *M. edulis* (Δ), reference site tunicates *C. inflata* (+), and IMTA farm site tunicates *C. inflata* (0).

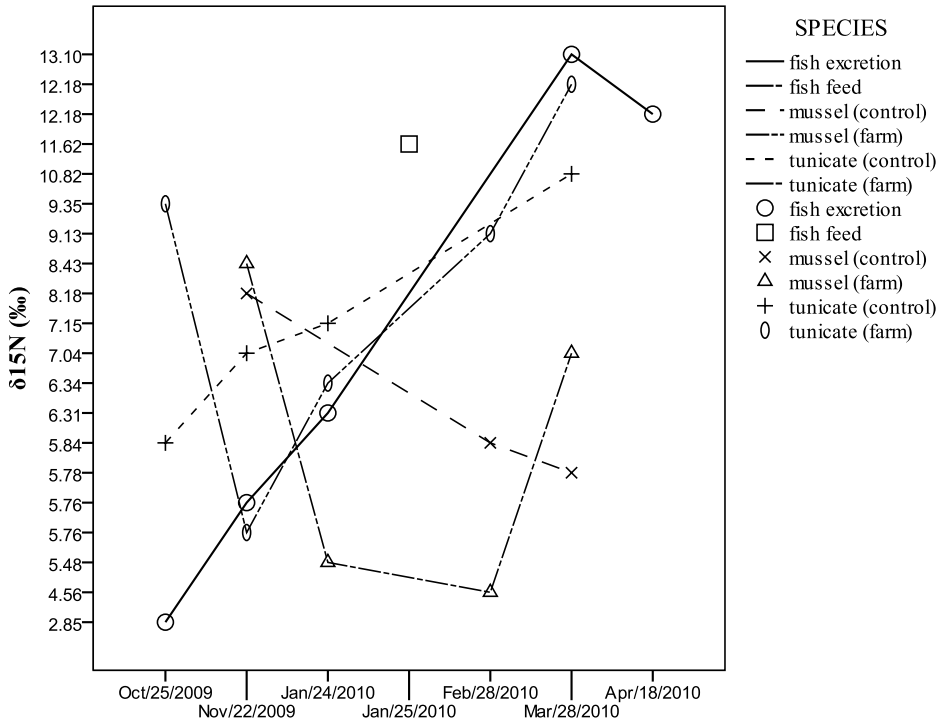


Figure 2.5 Mean temporal $\delta^{13}C$ variation (‰) for fish excretion (○), fish feed (□), reference site mussels *M. edulis* (x), IMTA farm site mussels *M. edulis* (Δ), reference site tunicates *C. inflata* (+), and IMTA farm site tunicates *C. inflata* (0).

ANOVA with Tukey's tests were performed to measure differences between $\delta^{13}C$ and $\delta^{15}N$ average signatures of each sample species at each sampling date. For *M. edulis*, there were few significant differences between sampling dates found in $\delta^{13}C$ values for IMTA values except for February and March 2010 samples (Table 2.1; df = 3, MS = 0.491, F = 4.064, p = 0.050). Tukey's test showed that $\delta^{15}N$ signatures from January 2010 were isotopically lighter than those sampled in February 2010 (Table 2.1; df = 3, MS = 0.539, F = 5.428, p = 0.025). There were no significant differences between sampling dates found in $\delta^{13}C$ and $\delta^{15}N$ values for tunicates *C. inflata* (Table 2.2; for $\delta^{13}C$: df = 4, MS = 5.071, F = 1.969, p = 0.192; for $\delta^{15}N$: df = 4, MS = 3.250, F = 1.889, p = 0.206). The greatest amount of temporal variation existed with each sample of fish excretion per month (for $\delta^{13}C$: df = 4, MS = 3.059, F = 14.684, p = 0.001; for $\delta^{15}N$: df = 4, MS = 2.996, F = 8.972, p = 0.005). Faecal samples from October 2009 and January 2010 were isotopically lighter in $\delta^{13}C$ than all other months. For measurements of faecal

$\delta^{15}N$ signatures, samples from January 2010 were significantly isotopically lighter than all other sampling months except for October 2009.

2.4.5 Relative Contribution of IMTA-Derived Food Sources to Suspension Feeding Epibionts

One-way ANOVA with Tukey's test were used to produce multiple comparisons of the mean $\delta^{13}C$ and $\delta^{15}N$ signatures of the potential food sources analyzed (for ^{13}C: df = 2, MS = 0.109, F = 0.078, p = 0.925; for ^{15}N: df = 2, MS = 51.725, F = 34.216, p = 0.000). The results showed that $\delta^{13}C$ signatures from all food source samples were not statistically different; all $\delta^{15}N$ signatures were not significantly different except for the marine POM isotopic average. IsoSource estimates of relative contributions of C and N to the diets of IMTA sampled *M. edulis* and *C. inflata* are listed in Table 2.5 (% ranges). For the diet of *M. edulis*, fish feed is the most dominant (47 – 100 %), whereas for the diet of *C. inflata*, marine POM is the most dominant food source (91 – 100 %). The food sources with the least impact on the diets of both consumers were marine POM for *M. edulis* (0 – 4 %) and both fish feed and fish faeces for *C. inflata* (0 – 9 % and 0 – 7 %, respectively).

Table 2.5 Carbon and nitrogen proportions (%) and average $\delta^{13}C$ and $\delta^{15}N$ signatures of possible food sources (marine particulate organic matter [POM], fish feed, and fish faeces) for suspension feeding epibionts (*M. edulis*, *C. inflata*). C and N contributions (%) are calculated values generated by software IsoSource (Phillips & Gregg 2003). Values with the same superscript letter are not significantly different ($p < 0.05$).

Food sources	**Relative range of C and N contributed to *M. edulis* (%)**	**Relative range of C and N contributed to *C. inflata* (%)**	**Ave. $\delta^{13}C$ (‰)**	**Ave $\delta^{15}N$ (‰)**
POM	0 – 4	91 – 100	-21.55^{a}	5.27^{b}
Fish feed	47 – 100	0 – 9	-21.62^{a}	10.48^{c}
Fish faeces	0 – 49	0 – 7	-21.45^{a}	11.70^{c}

Quantitative estimates of feasible relative contributions of food sources to IMTA-site sampled consumers are represented in Figure 2.6. According to the three-source mixing

model, estimates of proportional contribution of POM averaged 73.3 % in brooding transparent tunicates and 10.0 % in blue mussels. Estimates of proportional contribution of fish excretion averaged 84.3 % for blue mussels and 15.6 % brooding transparent tunicates. The estimated proportional contribution of fish feed was averaged to be 11.1 % for brooding transparent tunicates and 5.7 % for blue mussels.

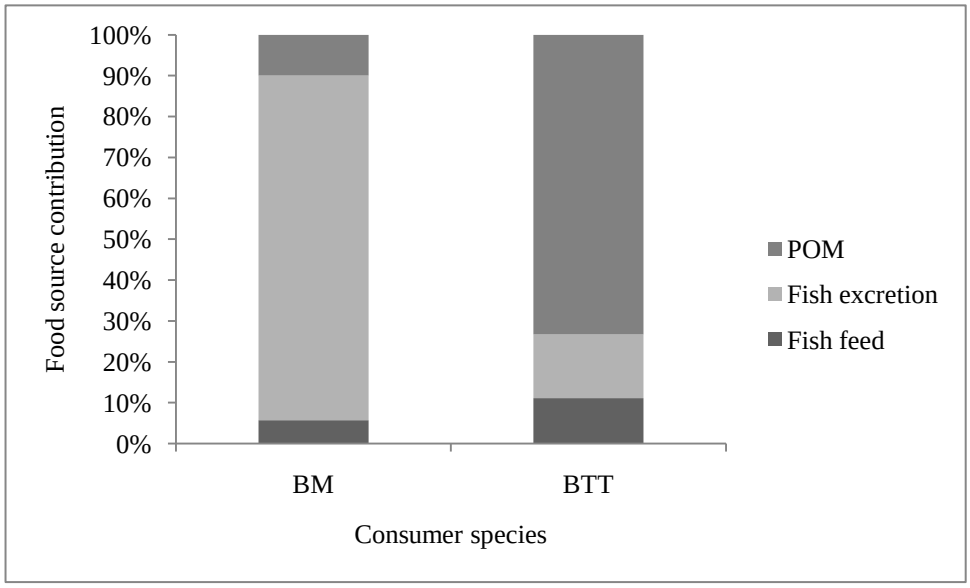


Figure 2.6 Histogram of interspecific variability of average relative contributions (%) of food sources for each sampled consumer species as estimated by 3-sources mixing model software IsoSource version 1.3.1. Food sources include particulate organic matter (POM), fish excretion and fish feed. Abbreviations of consumer species are BM, blue mussel (*Mytilus edulis*), and BTT, brooding transparent tunicate (*Corella inflata*).

The dual isotope plot (Fig. 2.7) displays average $\delta^{13}C$ and $\delta^{15}N$ signatures for all IMTA site samples (*M. edulis*, *C. inflata*, fish feed and fish excretion) and marine POM values taken from the literature review. ^{13}C values for the suspension feeding consumers appear spatially removed from all three potential food sources—much more enriched than what well-documented levels of carbon-enrichment suggest if IMTA farm-derived matter and POM were the only contributing sources. $\delta^{13}C$ of *M. edulis* were significantly different from all food sources as revealed by Tukey tests (df = 4, MS = 21.606, F =

12.942, p = 0.000). The same consensus is met when accounting for the 1 ‰ carbon enrichment value.

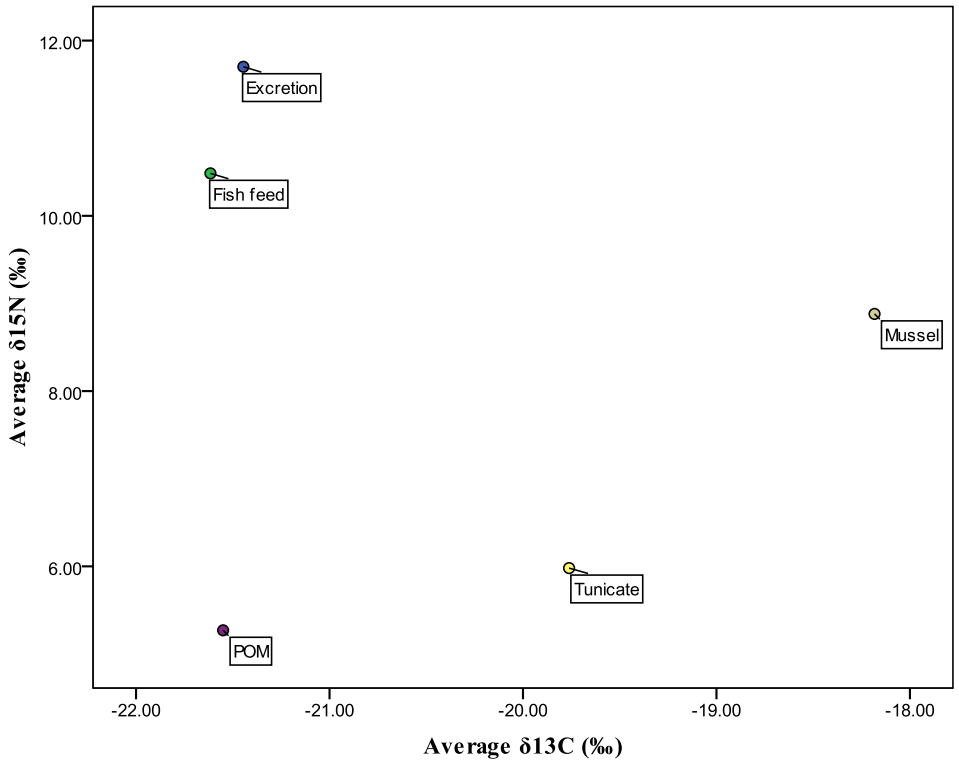


Figure 2.7 Dual isotope plot of mussel *M. edulis*, tunicate *C. inflata*, and potential food sources (fish feed, fish excretion and marine POM). Results represent averages. POM measurements were gathered from previously published studies (see Table 4). Note: consumer (mussel and tunicate) $\delta^{13}C$ and $\delta^{15}N$ signatures were not corrected to account for trophic fractionation.

2.5 Discussion

Possible competition between adjacent epibiont biofouling organisms and intended extractives has not been thoroughly investigated within integrated multi-trophic aquaculture (IMTA) facilities. Elucidating ecological linkages within IMTA systems may in turn lead to better understanding carrying capacities of intended extractives in order to benefit from enhanced harvest and ecological sustainability. In the present study, stable carbon (^{13}C) and nitrogen (^{15}N) isotopes from mussel *Mytilus edulis* tissues sampled at the IMTA site are statistically more significantly enriched than those found in mussel

tissues from the reference site. No statistical difference was found between fish feed and farmed fish faeces in terms of their average $\delta^{13}C$ and $\delta^{15}N$ isotopic values throughout the sampling period. For food web analyses, minimal shifts in carbon isotopes (~1 ‰) between consumer tissues and their food sources make it less suitable than nitrogen isotopes, which demonstrate predictable enrichment values of up to 3.5 ‰, for delineating and recreating food webs (Fry & Sherr 1984; Owens 1987). Generally, it has been tested that the main diet of wild mussels *M. edulis* is marine POM, the isotopic carbon and nitrogen fractionation values employed by isotope mixing models and within the diets of *M. edulis* and tunicates *C. inflata* should be obtained by the following $\delta^{13}C_{consumer}$ - $\delta^{13}C_{POM}$ and $\delta^{15}N_{consumer}$ - $\delta^{15}N_{POM}$ formulas (Gao *et al*. 2006). The results from the reference site consumers sampled in this study were 1.65 ‰ and 2.65 ‰, respectively for *M. edulis*, and 1.94 ‰ and 0.95 ‰, respectively for *C. inflata*. My findings do not show strong support for the 1 ‰ carbon and 3 ‰ nitrogen isotopic fractionation value. Given the absence of *in situ* data for POM, values taken from previously published literature were employed to represent an estimate of what reference site measurements of food sources could be in this particular geographic region. Since the average $\delta^{13}C$ measurement for marine POM was statistically similar to those of both fish feed and fish faecal material, multiple stable isotopic comparisons become essential to provide better resolution of food source contributions to consumers.

2.5.1 Inter- and Intraspecific Competition

Tunicates *Corella inflata* from the IMTA site displayed both stable carbon and nitrogen isotopic signatures that were not statistically different from those extracted from the reference site. Furthermore, *C. inflata* from both sampling sites, collectively, were significantly isotopically lighter in $\delta^{15}N$ than those signatures from all samples of *M. edulis* (IMTA farm and reference sites). This was unexpected considering *C. inflata* and *M. edulis* were sampled from the same aquaculture nets within each sampling site. Despite belonging to the same active suspension feeding guild as *M. edulis, C. inflata* displayed a consistent signature between sites, unlike mussel. Dubois *et al*. (2007) found similar results but with another ascidian species, the sea squirt *Ascidiella aspersa*. They suggested that *A. aspersa* may not feed continuously like other suspension feeding

species and instead filter feed at opportune times and/or conditions. The study further proposes that perhaps *A. aspersa* consumes fewer inorganic particles; feeding only when hydrologic conditions are stable (*ie*. during high tide) rather than during high velocity ebb and flow tides. Increased currents may suspend inorganic sedimentary matter that may interfere with feeding. Unlike the Dubois *et al*. (2007) study, the ascidian species (*C. inflata*) in the present study displayed the greatest intraspecific variability—particularly within the IMTA site—owing possibly to greater selection of potential food sources as compared to those sampled at the reference site. Dubois *et al*. (2007) suggests their ascidian *A. aspersa* are less capable of trophic plasticity. This study suggests otherwise for *C. inflata*. Perhaps the difference between both ascidian species could be due to the fact that hydrodynamic conditions within the IMTA site are relatively constant, including a constant supply of aquaculturally derived food sources. In contrast, *M. edulis* from the IMTA site measured the smallest degree of intraspecific variability which suggests low trophic plasticity.

Co-occurring suspension-feeding species *M. edulis* and *C. inflata* sampled within the IMTA site were found to have statistically different carbon and nitrogen isotopic signatures, despite having recruited on the same shellfish nets. Aside from *A. aspersa*, Dubois *et al*. (2007) measured isotopic signatures of several species of common suspension-feeding, biofouling epibionts (*ie*. *Pomatoceros lamarcki*, *Lanice conchilega*, *Mytilus edulis*, and *Elminius modestus*) and found they were not statistically similar to those of nearby cultivated oysters *Crassostrea gigas*, thereby also concluding that these associated species likely do not compete for food. IMTA-site samples of *M. edulis* displayed little to no overlap with IMTA-sampled *C. inflata* in both carbon and nitrogen isotopic signatures, demonstrating that they likely also do not for nutrients. Results from the IsoSource output confirm this as the diets of both IMTA sampled *M. edulis* and *C. inflata* were dominated by different potential food sources, fish feed and marine POM, respectively. These data lend support for the use of *M. edulis* as both harvestable extractives and as biofilters of farm-generated pollution from IMTA projects.

2.5.2 Relative Contribution of IMTA-Derived Food Sources to Suspension Feeding Epibionts

There could be several ways to explain why the $\delta^{13}C$ signatures of both solitary epibiont species measured significantly different from potential food sources. Two of the main assumptions by which stable isotopes analysis are based are (1) the number of potential food sources is limited; and (2) the identification of each food source is made possible because each possesses a different isotopic signature (Yokoyama & Ishihi 2007). Despite predictable levels of enrichment displayed by the isotopic values for ^{15}N, the dual isotope plot (Fig. 2.6) gives reason to believe that there may be one or more additional food sources yet to be identified or that food consumption is occurring at variable proportions (Dubois *et al*. 2007). Only IMTA-derived organic uneaten fish feed and farmed sablefish excretion were directly examined. In the case of this study other potential food sources could be benthic microalgae, macroalgae, terrestrial derived carbon washed into the ocean, and resuspended detrital material.

Other studies have demonstrated success in using $\delta^{13}C$ signatures for evaluating 3 or more food sources using isotopic mixing models (*eg*. Mazzola & Sarà 2001; Dubois *et al*. 2007; Yokoyama & Ishihi 2007). Despite providing a valuable baseline with which to elucidate trophic relationships, isotopic mixing models should be approached with caution. Several models make varying degrees of assumptions, consequently resulting in contradictory calculations (Caut *et al*. 2008). Since many mixing models are limited to how many isotope values (*n*, typically 2) and to how many organic matter sources can be computed (typically $n + 1$), outcomes of proportion of contribution to consumer diets may be overestimated because only primary sources assumed to be the most important are included (Phillips 2001; Benstead *et al*. 2006). As a consequence, reconstruction and further understanding of a food web structure may not produce accurate results.

Unlike the present study that separates uneaten fish feed from fish faeces, Lefebvre *et al*. (2000) distinguished integrated aquaculture effluent into two different organic forms, detrital matter (*ie*. fish faeces or uneaten fish feed) and dissolved excreted products transformed to living cells of phytoplankton via regenerated primary production.

Results of controlled food quality experiments (including pre-ingestive processes and absorption efficiency) found that diatoms (*Skeletonema costatum*) were preferentially ingested as compared to fish faeces when farmed Pacific oysters (*Crassostrea gigas*) were offered a mixed diet. These results not only indicate the necessity of measuring different feeding responses as a result of distinguishing different aquaculture effluent sources, but they also support the hypothesis that detrital waste generated from aquaculture can contribute to the growth of bivalve species.

Recently Reid *et al*. (2010) found lower than expected mean values for fish faecal organic particulates and absorption efficiency (AE) among *M. edulis* grown adjacent to Atlantic salmon (*Salmo salar*) in an IMTA system. They proposed that these results are linked to periods of significant inorganic particle plumes, such as silt fluxes, at their water sampling stations. Furthermore, they suggest that perhaps these samples were not directly taken within the exiting plume of the salmon cages. The results from this study may also draw similar conclusions. Additionally, it could also be that exiting plumes could change direction and depth given changes in seasonal current flow rates, water temperature, salinity, turbidity, and other abiotic variables. Further seasonal investigation into these variables adjacent to the sablefish cages is encouraged.

2.5.3 Spatial and Temporal Isotopic Shifts

In addition, possible large spatial and temporal shifts evidenced by the isotopic signatures of primary producers, like phytoplankton, can skew attempts to quantify their contribution to the diets of primary consumers if not taken into account (Melville & Connolly 2003). Studies have linked significant seasonal fluctuations of isotopic signatures of consumers with those of their primary producing prey (Riera & Richard 1997; Rolff 2000). In a nearby 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, and compared them with various measurements of environmental variables (*eg*. salinity, total particulate matter, particulate organic matter, particulate inorganic matter, carotenoids, chlorophylls *a*, *b*, and *c* concentrations, pH, dissolved oxygen, and water temperature). They found that instantaneous growth in *C. gigas* was

strongly correlated with chlorophyll *b*, which is indicative of the influence of marine nanoplankton to their diets. Highest concentrations of chlorophyll *b* occurred during the summer months which correlated with highest shell growth rates of *C. gigas*. The present study was conducted primarily during winter and spring months (October to April) when chlorophyll *b* levels, total particulate matter, and water temperature are relatively low in this region and may account for why fish feed, followed by fish faeces, were the dominant food sources demonstrated by the mixing model. Overall, the monthly fluctuations of trace carbon and nitrogen signatures demonstrated in this study (Figs. 2.4 & 2.5) are likely characterized by the dynamic nature of the marine environment. As the study approached spring, $\delta^{13}C$ signatures of all samples studied became less enriched—possibly owing to an increasing production of marine phytoplankton.

The results of the present study provides no apparent evidence for resource competition between biofouling epibiont species *M. edulis* and *C. inflata* and substantiate the feasibility of rearing blue mussels *M. edulis* as organic extractive species within an IMTA system during winter months. This is indicated by contributions of IMTA system effluent trace ^{13}C and ^{15}N signatures within their tissues. Future research into the adjacent biotic and abiotic changes and over longer temporal periods are highly encouraged within this particular region as food source portioning may alter seasonally. Analysis of additional producer and consumer organisms and other isotopes are also recommended.

Literature Cited

Bayne BL, Widdows J, Newell RIE (1977). Physiological measurements on estuarine bivalve mollusks in the field. In: *Biology of Benthic Organisms* (Ed. Keegan BF, Ceidigh PO, and Boaden PJS), Oxford Pergammon Press, pp. 57—68.

Benstead JP, March JG, Fry B, Ewel KC, Pringle CM (2006). Testing IsoSource: stable isotope analysis of a tropical fishery with diverse organic matter sources. *Ecology* 87: 326—333.

Bosley KL, Wainwright SC (1999). Effects of preservatives and acidification on the stable isotope ratios (^{15}N:^{14}N, ^{13}C:^{12}C) of two species of marine animals. *Can J Fish Aquat Sci* 56(11): 119—127.

Brown JR (1988). Multivariate analysis of the role of environmental factors in seasonal and site-related growth variation in the Pacific oyster *Crassostrea gigas*. *Mar Ecol Prog Ser* 45: 225—236.

Bunting SW (2008). Horizontally integrated aquaculture development: exploring consensus on constraints and opportunities with a stakeholder Delphi. *Aquaculture International* 16: 153—169.

Bustamante RH, Branch GM (1996). The dependence of intertidal consumers of kelp-derived organic matter on the west coast of South Africa. *J Exp Mar Biol Ecol* 196: 1—28.

Caut S, Angulo E, Courchamp F (2008). Caution on isotopic model use for analysis of consumer diet. *Can J Zool* 86: 438—445.

Chopin T (2006). Integrated multi-trophic aquaculture. What it is, and why you should care…and don't confuse it with polyculture. *Northern Aquaculture* 12: 4.

Connell JH, Slatyer RO (1977). Mechanisms of succession in natural communities and their role in community stability and organization. *Am Nat* 111: 1119—1144.

Dalsgaard J, St John M, Kattner G, Muller-Navarra D, Hagen W (2003). Fatty acid trophic markers in the pelagic marine environment. *Adv Mar Biol* 46: 225—340.

Dame RF (1996). *Ecology of Marine Bivalves: An Ecosystem Approach*. CRC Press, Boca Raton.

Dauby P (1989). The stable isotope ratios in benthic food webs of Gulf of Calvi, Corsica. *Cont Shelf Res* 9: 181—195.

Deegan LA, Garritt RH (1997). Evidence for spatial variability in estuarine food webs. *Mar Ecol Prog Ser* 147: 31—47.

De Niro MJ, Epstein S (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 42: 495—506.

Dubois S, Orvain F, Marin-Léal JC, Ropert M, Lefebvre S (2007). Small-scale spatial variability of food partitioning between cultivated oysters and associated suspension-feeding species, as revealed by stable isotopes. *Mar Ecol Prog Ser* 336: 151-160.

Essington T, Beaudreau AH, Wiedenmann J (2006). Fishing through marine food webs. *Proc Natl Acad Sci*. 103: 3171—3175.

Folke C, Kautsky N (1989). The role of ecosystems for a sustainable development of aquaculture. *Ambio* 18:234—243.

Fry B, Sherr EB (1984). $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contrib Mar Sci* 27: 13—47.

Gannes LZ, O'Brien DM, del Rio CM (1997). Stable isotopes in animal ecology; assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78: 1271—1276.

Gao QF, Shin PKS, Guang-Hui L, Shi-Ping C, Cheung SG (2006). Stable isotope and fatty acid evidence for uptake of organic waste by green-lipped mussels *Perna viridis* in a polyculture fish farm system. *Mar Ecol Prog Ser* 317: 273—283.

Goering J, Alexander V, Haubenstock N (1990). Seasonal variability of stable carbon and nitrogen isotope ratios of organisms in a North Pacific Bay. *Est Coast Shelf Sci* 30: 239—260.

Greene CH, Schoener A, Corets, E (1983). Succession on marine hard substrata: the adaptive significance of solitary and colonial strategies in temperate fouling communities. *Mar Ecol Prog Ser* 13: 121—129.

Hargrave BT, Duplisea DE, Pfeffer E, Wildish DJ (1993). Seasonal changes in benthic fluxes of dissolved oxygen and ammonium associated marine cultured Atlantic salmon. *Mar Ecol Prog Ser* 96: 249—257.

Hobson KA, Welch HE (1992). Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. *Mar Ecol Prog Ser* 84:9—18.

Hobson KA, Piatt JF, Pitocchelli J (1994). Using stable isotopes to determine seabird trophic relationships. *J Anim Ecol* 63: 786—798.

Jennings S, Renones O, Morales-Nin B, Polunin NVC, Moranta J, Coll J (1997). Spatial variation in the 15N and 13C stable composition of plants, invertebrates and fishes on Mediterranean reefs: implications for study of trophic pathways. *Mar Ecol Prog Ser* 146: 109—116.

Jones TO, Iwama GK (1991). Polyculture of the pacific oyster, *Crassostrea gigas* (Thunberg), with Chinook salmon, *Oncorhynchus tshawytscha*. *Aquaculture* 92: 313—322.

Kaehler S, Pakhomov EA (2001). Effects of storage and preservation on the $\delta^{13}C$ and $\delta^{15}N$ signatures of selected marine organisms. *Mar Ecol Prog Ser* 219: 299—304.

Kaehler S, Pakhomov EA, McQuaid CD (2000). Trophic structure of marine food web at the Prince Edward Islands (Southern Ocean) determined by $\delta^{13}C$ and $\delta^{15}N$ analysis. *Mar Ecol Prog Ser* 208: 13—20.

Kamermans P (1994). Similarity in food source and timing of feeding in deposit- and suspension-feeding bivalves. *Mar Ecol Prog Ser* 104: 63—75.

Karakasis I, Hatziyanni E, Tsapakis M, Plaiti W (1999). Benthic recovery following cessation of fish farming: a series of successes and catastrophes. *Mar Ecol Prog Ser* 184: 205—218.

Kharlamenko VI, Kiyashko SI, Imbs AB, Vyshkvartzev DI (2001). Identification of food sources of invertebrates from the seagrass Zostera marina community using carbon and sulfur stable isotope ratio and fatty acid analyses. *Mar Ecol Prog Ser* 220: 103—117.

Kutti T, Ervik A, Hansen PK (2007). Effects of organic effluents from a salmon farm on a fjord system. I. Vertical export and dispersal processes. *Aquaculture* 262: 367—381.

Lajtha K, Michener R (2007). *Stable Isotopes in Ecology and Environmental Science*, 2nd ed. Wiley-Blackwell Publishing, pp. 592.

Lamb A, Hanby BP (2005). *Marine Life of the Pacific Northwest: A Photographic Encyclopedia of Invertebrates, Seaweeds and Selected Fishes*. Harbour Publishing, BC., pp. 398.

Lambert G, Lambert CC, Abbott DP (1981). *Corella* species in the American Pacific Northwest: distinction of *C. inflata* Huntsman, 1912 from *C. willmeriana* Herdman, 1898 (Ascidiacea, Phlebobranchia). *Can J Zool* 59: 1493—1504.

Larsson AM (1985). Blue mussel sea farming-effects on water quality. *Vatten* 41: 218—224.

Lefebvre S, Barillé L, Clerc M (2000). Pacific oyster (*Crassostrea gigas*) feeding responses to a fish-farm effluent. *Aquaculture* 187: 185—198.

Lehane C, Davenport J (2002). Ingestion of mesozooplankton by three species of bivalves: *Mytilus edulis*, *Cerastoderma edule* and *Aequipecten opercularis*. *J Mar Biol Assoc UK* 82: 615—619.

Lesser MP, Shumway SE, Cucci T, Smith J (1992). Impact of fouling organisms on mussel rope culture: interspecific competition for food among suspension-feeding invertebrates. *J Exp Mar Biol Ecol* 165: 91—102.

Marion A, Harvey M, Chabot D, Brêthes J-C (2008). Feeding ecology and predation impact of the recently established amphipod, *Themisto libellula*, in the St. Lawrence marine system, Canada. *Mar Ecol Prog Ser* 373: 53—70.

Martínez-Porchas M, Martínez-Cordova LR, Porchas-Cornejo, MA, López-Elías, JA (2010). Shrimp polyculture: a potentially profitable, sustainable, but uncommon aquacultural practice. *Rev Aquaculture* 2(2): 73—85.

Mazzola A, Sará G (2001). The effect of fish farming organic waste on food availability for bivalve mollusks (Gaeta Gulf, Central Tyrrhenian, MED): stable isotopic analysis. *Aquaculture* 192: 361—379.

Melville AJ, Connolly RM (2003). Spatial analysis of stable isotope data to determine primary sources of nutrition for fish. *Oecologia* 136: 499—507.

Michener RH, Schell DM (1994). Stable isotope ratios as tracers in marine aquatic food webs. In: Lajtha K, Michener RH (eds) *Stable isotopes in ecology and environmental science*. Blackwell Scientific Publications, London, pp. 138—157.

Nadon M-O, Himmelman JH (2006). Stable isotopes in subtidal food webs: Have enriched carbon ratios in benthic consumers been misinterpreted? *Limnol Oceanogr* 51(6): 2828—2836.

Naylor R, Goldburg RJH, Primavera JH, Kautsky N, Beveridge MCM, Clay J (2000). Effect of aquaculture on world fish supplies. *Nature* 405: 1017—1024.

Neori A, Chopin T, Troell M, Buschmann AH, Kraemer GP, Halling C, Shpigel M, Yarish C (2004). Inegrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture* 231: 361—391.

Neori A, Troell M, Chopin T, Yarish C, Critchley A, Buschmann AH (2007). The need for a balanced ecosystem approach to blue revolution aquaculture. *Environment* 49: 36—43.

Owens NJP (1987). Natural variations in ^{15}N in the marine environment. *Adv Mar Biol* 24: 389—451.

Pearson TH, Rosenberg R (1978). Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanogr Mar Biol Rev* 16: 229—311.

Peterson BJ, Fry B (1987). Stable isotopes in ecosystem studies. *Annu Rev Ecol Syst* 18: 293—320.

Phillips DL (2001). Mixing models in analyses of diet using multiple stable isotopes: a critique. *Oecologia* 127: 166—170.

Phillips DL & Gregg JW (2003). Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136: 261—269.

Pinnegar JK, Polunin NVC (1999). Differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. *Funct Ecol* 225—231.

Polis GA, Anderson WB, Holt RD (1997). Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annu Rev Ecol Syst* 28: 289—316.

Prins TC, Smaal AC, Dame RF (1998). A review of feedbacks between bivalve grazing and ecosystem processes. *Aquat Ecol* 31: 349—359.

Quan W, Fu C, Jin B, Luo Y, Li B, Chen J, Wu J (2007). Tidal marshes as energy sources for commercially important nekton organisms: stable isotope analysis. *Mar Ecol Prog Ser* 352: 89—99.

Reid GK, Liutkus M, Bennett A, Robinson SMC, MacDonald B, Page F (2010). Absorption efficiency of blue mussels (*Mytilus edulis* and *M. trossulus*) feeding on Atlantic salmon (*Salmo salar*) feed and fecal particulates: Implications for integrated multi-trophic aquaculture. *Aquaculture* 299: 165—169.

Riera P, Richard P (1997). Temporal variation of δ13C in particulate organic matter and oyster Crassostrea gigas in Marennes-Oléron Bay (France): effect of freshwater inflow. *Mar Ecol Prog Ser* 147: 105—115.

Riera P, Stal LJ, Nieuwenhuize J (2002). $\delta^{13}C$ vs $\delta^{15}N$ of co-occurring molluscs within a community dominated by *Crassostrea gigas* and *Crepidula fornicata* (Oosterschelde, The Netherlands). *Mar Ecol Prog Ser* 240: 291—295.

Rolff C (2000). Seasonal variation of δ13C and δ15N of size-fractionated plankton at a coastal station in the northern Baltic proper. *Mar Ecol Prog Ser* 203: 47—65.

Sará G, Scilipoti D, Milazzo M, Modica A (2004). Effects of fish farming waste to sedimentary and particulate organic matter in a southern Mediterranean area (Gulf of Castellammare, Sicily): a multiple stable isotope study ($\delta^{13}C$ and $\delta^{15}N$). *Aquaculture* 234(1-4): 199—213.

Sarà G, Zenone A, Tomasello A (2009). Growth of *Mytilus galloprovincialis* (mollusca, bivalvia) close to fish farms: a case of integrated multi-trophic aquaculture within Tyrrhenian Sea. *Hydrobiologica* 636: 129—136.

Shpigel M, Blaylock RA (1991). The pacific oyster, *Crassostrea gigas*, as a biological filter for a marine fish aquaculture pond. *Aquaculture* 92: 187—197.

Shpigel M, Gasith A, Kimmel E (1997). A biomechanical filter for treating fish-pond effluents. *Aquaculture* 152: 103—117.

Shpigel M, Neori A, Gordin H (1991). Oyster and clam production in the outflow water of marine fish pond in Israel. *EAS Spec Publ* 14: 295.

Shpigel M, Neori A, Popper DM, Gordin H (1993b). A proposed model for environmentally clean land-based culture of fish, bivalves and seaweeds. *Aquaculture* 117: 115—128.

Simenstad CA, Wissmar RC (1985). $\delta^{13}C$ evidence of the origins and fates of organic carbon in estuarine and nearshore food webs. *Mar Ecol Prog Ser* 22(2): 141—152.

Stirling HP, Okumus I (1995). Growth and production of mussels (*Mytilus edulis* L.) suspended at salmon cages and shellfish farms in two Scottish sea lochs. *Aquaculture* 134: 193—210.

Sutherland TF, Martin AJ, Levings CD (2001). Characterization of suspended particulate matter surrounding a salmonid net-pen in the Broughton Archipelago, British Columbia. *ICES* 58: 404—410.

Sweeting CJ, Jennings S, Polunin NVC (2005). Variance in isotopic signatures as a descriptor of tissue turnover and degree of omnivory. *Funct Ecol* 19: 777—784.

Troell M, Halling C, Neori A, Chopin T, Buschmann AH, Kautsky N, Yarish C (2003). Integrated mariculture: asking the right questions. *Aquaculture* 226: 69—90.

Weston DP (1990). Quantitative examination of macrobenthic community changes along an organic enrichment gradient. *Mar Ecol Prog Ser* 61: 233—244.

Widdows J, Fieth P, Worrall CM (1979). Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. *Mar Biol* 50: 195—207.

Wu RSS (1995). The environmental impact of marine fish culture: Towards a sustainable future. *Mar Poll Bull* 31: 159—166.

Wu RSS, Lam KS, Mackay DW, Lau TC, Yam V (1994). Impact of marine fish farming on water quality and bottom sediment: a case study in the sub-tropical environment. *Mar Environ Res* 38: 115—145.

Ye L, Ritz DA, Fenton GE, Lewis ME (1991). Tracing the influence on sediments of organic waste from a salmonid farm using stable isotope analysis. *J Exp Mar Biol* 145: 161—174.

3.0 TEMPORAL AND SPATIAL VARIATION OF $\delta^{13}C$ AND $\delta^{15}N$ STABLE ISOTOPES OF EXTRACTIVE AND BIOFOULING ORGANISMS WITHIN AN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEM

3.1 Abstract

Aquaculture facilities emit large quantities of waste that may subsidize nearby epibiont filter and deposit feeders providing both environmental and economic benefits. However, biofouling organisms form communities within and adjacent to aquaculture facilities that may pose as potential competitors to organic and inorganic extractives intended for harvest. Seasonal fluctuations of biotic and abiotic processes may influence food source partitioning of organisms within and adjacent to aquaculture facilities which can alter monitoring and carrying capacity measurements of harvestable species. Stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) were employed to seasonally characterize and reconstruct a food web within an integrated multi-trophic aquaculture (IMTA) facility in Kyuquot Sound, BC. It was predicted that aquaculture-generated effluent would be detectable to varying degrees within the tissues of nearby intended extractives (blue mussels *Mytilus edulis*, sea urchins *Strongylocentrotus franciscanus*, Pacific scallops *Patinopectin yessoensis*, sugar kelp *Saccharina latissima*) and biofouling organisms (broadbase tunicate *Cnemidocarpa finmarkiensis*, brooding transparent tunicates *Corella inflata*, hairy tunicate *Boltenia villosa*, sea cucumber *Parastichopus californicus*, *Rhodophyte* sp.). A multi-source mixing model was used to quantify the relative contribution of aquacultural waste to sample organisms. There was no statistically significant directional change detected in the entire food web across seasons. This is possibly due to the consistent supply of aquaculture-generated effluent that is assumed to maintain constant isotopic composition. Advanced food web knowledge within sustainable aquaculture programs can serve as a baseline when comparing food web dynamics in other related projects.

3.2 Introduction

Integrated multi-trophic aquaculture (IMTA) refers to the aquatic farming practice of utilizing the waste of one system as an nutrient input to one or more subsystems that permits greater overall efficiency of intended products (Barrington *et al*. 2009). IMTA facilities are designed to mimic natural ecosystems, where organic and inorganic by-products from one species, which would otherwise pollute the surrounding water column, are recycled by other intended species. These intended species, or extractives, represent different trophic levels linked within a natural food web (Chopin 2006). Like natural marine food webs, species placed within an IMTA facility share the same biological and chemical processes and are linked spatially through nutrient and energy subsidies transported by water. Each species is combined in appropriate proportions as to allow maximum harvest growth and environmental sustainability, which leads to optimal economic stability, biomitigation and impact reduction (Duarte *et al*. 2003; Barrington *et al*. 2009). Carrying capacity of aquaculture systems has been reviewed and investigated through mathematical modelling, with each varying in terms of complexity and number of factors calculated (Duarte *et al*. 2003; Ferreira *et al*. 2006; McKindsey *et al.* 2006). Temporal variation and presence of non-intended organisms represent factors rarely built into carrying capacity models regardless of the fact that natural marine trophic relationships are not static, with nutrient fluxes and resource partitioning shifting constantly.

Understanding nutrient supplies within marine food webs subsequently leads to better understanding of the diversity, density and biomass of secondary consumers (Vizzini & Mazzola 2003). Primary food sources of naturally occurring benthic organisms are mostly derived from macrophytes and particulate organic matter (POM) in the form of zoo- and phytoplankton. The relative quantities of food sources within oceanic ecosystems vary spatially and temporally depending on several abiotic factors, including tidal mixing processes, temperature and salinity fluxes (Hsieh *et al*. 2002; Vizzini & Mazzola 2006). These hydrological processes can lead to fluctuations of trophic interactions within food webs that change over various time scales. Within aquacultural related structures, studies have shown that uneaten fish feed and

fish/shellfish waste effluent serve as additional food sources to consider when elucidating food webs (Gao *et al*. 2006). In concert with naturally occurring primary producers, aquaculture effluent may or may not exist as the most preferred source of food for intended or unintended species within an IMTA facility at all times.

Understanding species within their food web contexts are useful for many applications, and require several methods, including gut content and stable isotopes analysis, in order to better understand these applications (Polis *et al*. 2004). These methods can be employed as tools towards resource management within aquaculture systems, such as calculating the amount of extractives, or harvestable lower trophic organisms (eg. shellfish, seaweed), required to assimilate effluent waste generated by other higher trophic organisms (eg. fish) within an integrated multi-trophic aquaculture (IMTA) system.

Stable isotopes analysis is useful for elucidating and reconstructing food webs. Stable isotopic $\delta^{13}C$ and $\delta^{15}N$ signatures, for example, exist in trace amounts within organic material (Peterson & Fry 1987). $\delta^{13}C$ signatures within organic tissues of consumers are slightly enriched compared to the levels that exist in their food source (DeNiro & Epstein 1978; Fry & Sherr 1984). This slight enrichment, or the difference between the isotopic signature of a consumer and its food source, is known as metabolic fractionation, and is averaged to be approximately 1—1.5 ‰ for marine invertebrate $\delta^{13}C$ signatures (DeNiro & Epstein 1978). Carbon ratios are considered ideal data for better understanding organic sources at the base of most food webs. This is because aquatic and marine plants often exhibit stable carbon ratios different from terrestrial plants, and this difference is attributed to differences in photosynthetic carbon fixation (Smith & Epstein 1971; Rau 1978). Since heavier nitrogen isotopes preferentially assimilate to animal tissues and lighter nitrogen isotopes are excreted, $\delta^{15}N$ signatures within tissues tend to show greater predictable fractionation (approximately 3 ‰ enrichment) relative to food sources, which proves more useful for the identification of trophic position within a food web (Michener & Schell 1994).

Most studies that employ the use of stable isotopes data for community-wide food web reconstruction traditionally create $\delta^{13}C$—$\delta^{15}N$ (or $\delta^{13}C$—$\delta^{34}S$) bi-plots with which to test hypotheses. Despite assisting to visualize the trophic niche space of sample individuals, inferences made about food web linkages are strictly qualitative in that each plot represents a given time period. This is particularly restrictive for better understanding temporal changes of these linkages (Schmidt *et al*. 2007). Recent studies have recognized the need to shift data treatments from qualitative to quantitative approaches to understanding food web dynamics (Schindler & Lubetkin 2004). For example, Laymen *et al*. (2007) analyzed six separate quantitative metrics using stable isotope data drawn from a Bahamanian tidal creek food web; however they still only address community-wide measures of trophic diversity and estimations of the extent of trophic redundancy. There are a growing number of studies that attempt to examine trophic shifts within food webs along timescales spanning from seasons to hundreds of years (Schmidt *et al*. 2007).

Circular statistical metrics can allow for explicit hypothesis testing with regards to food web relationships over time and space (Schmidt *et al*. 2007). Circular statistics involve the analysis of angular data which ranges from 0 to 360° or 0 to 2π radians (Batschelet 1981; Zar 1999; Schmidt *et al*. 2007). Unlike basing data on normal or Gaussian distributions, circular statistics bases hypothetical data on circular normal or von Mises distributions (Zar 1999). In order to do this, monthly $\delta^{13}C$ and $\delta^{15}N$ signatures for each species were averaged and measured for magnitude and directional change within food web space as coordinates (x, y). Directional change is calculated by measuring the difference between two values, or points, in trophic niche space ($\delta^{13}C$ – $\delta^{15}N$) that, for the present study, represent monthly averages (Schmidt *et al*. 2007). They represent both direction (angle of change, θ) and length (magnitude of change). All angles of change can also be averaged, and this represents the mean vector of change, each with a direction (mean angle, μ) and a length (r).. A parameter r equaling (or close to) 0 represents uniform distribution whereas the closer r gets to a value of 1, the more concentrated the data angles. As with Gaussian distributions, descriptive statistics such as standard deviation and variance can be calculated for circular data that represent trophic

niche space of organisms under study, representing normal von Mises distributions (Schmidt *et al*. 2007). Furthermore, as with Gaussian distributions, mean averages of angular data from von Mises distributions are assumed to represent entire populations analyzed in ecological niche studies.

Seasonal environmental fluctuations mean that availability of particular carbon and nitrogen sources can also vary seasonally. In winter months, run-off tends to be high and terrestrially derived organic matter, which is depleted in ^{13}C, may become more available to marine consumers (Darnaude *et al*. 2003). Diatoms have been measured to exhibit increased abundance at this time (Dauby *et al*. 1990). Additionally during this time, irradiance levels are at annual lows, which could also explain lower than average $\delta^{13}C$ isotopic values in primary producers (Grice *et al*. 1996; Vizzini & Mazzola 2003). Marine plants are able to use various sources of carbon (HCO_3^- and CO_2) and nitrogen (NO_3^- and NH_4^+), and these sources vary in their respective isotopic signatures (Hemminga & Mateo 1996; Michener & Schell 1994). During winter months, nitrates move to the surface by increased storm activity and hydrodynamic mixing. Primary producers use nitrate which is generally considered a more enriched form of nitrogen available at this time (Wada *et al*. 1975; Michener & Schell 1994). By summer, nitrate has largely become unavailable and primary producers shift to rely primarily on recycled ammonia, which has more depleted $\delta^{15}N$ signatures (Michener & Schell 1994). During summer, dinoflagellate abundance increases, which increase $\delta^{13}C$ values of POM due to their utilization of a different photosynthetic pathway than ^{13}C depleted diatoms (Dauby *et al*. 1990).

I hypothesize that due to the spatial proximity of intended extractives and biofouling communities to the IMTA facility, carbon and nitrogen signatures of these organisms should be predictably more enriched than signatures measured from IMTA-derived waste and fish feed. Furthermore, I hypothesize a slight seasonal variation in isotopic signatures of all measured organisms indicated by quantitatively measured directional change. The present study will propose to (a) present isotopic data of organisms and identify potential food sources sampled within an IMTA system and from the literature; to (b) use this isotopic data to reconstruct the food web structure with the IMTA system; to (c) detect

and measure degree of change of niche partitioning among sample groups over several months using a multi-source mixing model developed by Phillips and Gregg (2003); and to (d) employ circular statistical metrics outlined by Schmidt *et al*. (2007) in order to investigate any temporal or spatial changes to the marine food web. Elucidating food web structure over a year is hypothesized to reveal the extent of food web change within an IMTA facility and assist in guiding the further development and management of sustainable aquaculture in Kyuquot Sound, British Columbia.

3.3 Materials and Methods

3.3.1 Study Site

The IMTA study site is situated on the northwest region of Vancouver Island, British Columbia (50°3’10”N 127°18’45”W) just off Surprise Island, Kyuquot Sound and approximately 4.5 kilometers from the village of Kyuquot. The approximately 5 km^2 marine study area is characterized by circular tidal flow, with a depth that is mostly photic, at approximately 30 m. A nearby anadromous fish stream, British Creek, discharges relatively low flows fresh water into the site. Current flow rate is low and runs laterally through a series of seven sablefish cages (50 x 50 ft^2, 60 ft deep) seasonally ranging from approximately 4,500 to 10,000 fish per cage. The ocean current flows through to a series of 250 shellfish droplines spaced one metre apart and are deployed in a raft system that is approximately 14 metres across x 75 metres long. Each dropline has 12 tiers, the top of which is approximately 5 metres deep with each tier housing approximately 25 to 50 Pacific scallops (*Patinopectin yessoensis*). The current finally passes through a number of sugar kelp (*Saccharina latissima*) lines. The seafloor is dominated by a muddy substrate.

Figure 3.1 Map of Vancouver Island, Canada featuring study site at Surprise Island (indicated by arrow).

Figure 3.2 Satellite image of Surprise Island, BC at larger scale (indicated by arrow).

3.3.2 Sample Collection

Samples were collected monthly (May 2009 to April 2010) from the commercial-scale IMTA site. Within the system, sablefish *Anoplopoma fimbria* samples were obtained by Pacific SEA-Lab, proprietor of the IMTA facility; the detritivore species (sea cucumbers *Parastichopus californicus***,** sea urchins *Strongylocentrotus franciscanus*, ochre star *Pisaster ochraceous*), the bivalve species (blue mussels *Mytilus edulis*, scallops *Patinopecten yessoensis*), brooding transparent tunicates *Corella inflata*, hairy tunicates *Boltenia villosa*, and broadbase tunicates *Cnemidocarpa finmarkeinsis*, were retrieved from a series of adjacent shellfish rafts down-current from the fish cages. Macroalgal species (sugar kelp *Saccharina latissima*, broad-rib kelp *Pleurophycus gardneri*, and *rhodophyte* sp.) were sampled by hand from a series of lines also downstream from the facility. It was not always possible to collect each species each month. Sample collection was dependent upon organisms that were present within the system during collection periods (see Table 3.2 for all species collected and sampling dates).

All samples were dissected and processed in an on-site laboratory. Prior to dissection, samples were rinsed with distilled water. Only tissues with known high turnover rates and low variability were sampled, which include the liver of the sablefish (Perga & Gerdeaux 2005; Lajtha & Michener 2007) and whole soft bodies (dissected from the shell and operculae of bivalves) of the blue mussels, tunicates, and anemones using a scalpel or surgical blade (Dubois *et al*. 2007). The digestive glands of the scallops were assayed, as these tissues better reflect their current diets, exhibiting faster turnover rates (Aya & Kudo 2007; Lorraine *et al*. 2002). Somatic tissue, such as gonads for sea urchins *S. franciscanus*, were analyzed (Berger & Jelinski 2008) as well as sections of the body wall in sea cucumbers (Iken *et al*. 2001). Whole seaweed fronds were used for isotopic analysis. Sampling tissues with high turnover rates ensure that isotopic values can be biased towards feeding patterns of the recent past, which is more suitable for a short term analysis. Many studies have demonstrated that different tissues reflect diet at different rates (Sarakinos *et al*. 2002). Since ^{13}C materials are highly enriched with inorganic carbonates, tissues were soaked in 5% HCl in order to remove them (Simenstad

& Wissmar 1985; Bosley & Wainright 1999; Pinnegar & Polunin 1999; Kaehler & Pakhomov 2001). Dissected tissue samples, fish faeces, as well as samples of commercial fish feed (Taplow aquafeed) were oven-dried at 60 ºC for a minimum of 24 h, then ground to a fine powder using a pestle and mortar (Sarà *et al*. 2004). 1 mg samples of all tissues were weighed in tin containers (6 x 4 mm and 5 x 8 for oily samples) in preparation for sample continuous flow isotope ratio spectrometry (CF-IRMS) analysis.

3.3.3 Isotopic Analysis

Isotopic measurements were performed at the University of California—Davis Stable Isotope Facility. CF-IRMS analysis was completed using a Europa Hydra 20/20 continuous-flow isotope ratio mass spectrometer. Analytical error of $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$) as:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where X is either carbon (C) or nitrogen (N) and R is the ratio of heavy to light stable isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). Triplicate samples were analyzed haphazardly and resulted in an analytical reproducibility of 0.98 ‰ for $\delta^{13}C$ and 0.45 ‰ for $\delta^{15}N$.

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 (Simenstad & Wissmar 1985; Riera & Richard 1997; Kang *et al*. 1999; Vizzini & Mazzola 2002; Vizzini & Mazzola 2003). To better understand seasonal variability, tissues exhibiting low variability and

high turnover rates were selected for the present study. If data is unknown, whole bodies were sampled.

3.3.4 Literature Review for $\delta^{13}C$ and $\delta^{15}N$ of Marine Particulate Organic Matter

Studies were reviewed in order to determine the importance of marine particular organic matter (POM) to the diet of mussel and tunicate sample group sampled within the IMTA site. This provided 4 measurements of $\delta^{13}C$ and 3 measurements of $\delta^{15}N$ for POM within similar geographic regions to Kyuquot Sound, BC. Table 3.1 shows the isotopic values from the literature.

Table 3.1 Results of literature review of average marine particulate organic matter (POM) carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values from geographic regions similar to that of the present study site. Since *in situ* marine POM data is missing from this analysis, this data are hypothetical and represent possible non-IMTA effluent sources contributing to consumer diets.

Geographic region of POM sample	**$\delta^{13}C$ (‰) Mean ± S.D.**	**$\delta^{15}N$ (‰) Mean ± S.D.**	**Reference**
Auk Bay, Alaska	-20.3 ± 0.9	2.7	Goering *et al*. 1990
Fritz Cove, Alaska	-20.3 ± 0.2	6.3	Goering *et al*. 1990
Broughton Archipelago, BC	-24.0 ± 1.0	n.d.	Sutherland *et al*. 2001
Barkley Sound, BC	-21.6 ± 0.2	6.8 ± 0.1	Hobson *et al*. 1994

3.3.5 Isotope Mixing Model for Multiple Food Sources

Many studies use isotopic mixing models to evaluate the proportion of each food source to the diet of various consumers in marine systems (Bustamante *et al*. 1996; Kaehler *et al*. 2000; Gao *et al*. 2006; Nadon & Himmelman 2006). The present study has recognized fish feed, fish excretion, macroalgae, and marine POM as potential food sources for marine extractives and biofouling epibionts found within and adjacent to the IMTA system. Accordingly, I sought to determine the percent contribution of these IMTA-derived primary producers along with POM values within consumers using the following multi-source system isotope mixing model described by Phillips (2001):

$$(\delta^{13}C'_A - \delta^{13}C_D)[C]_A f_{A,BM} + (\delta^{13}C'_B - \delta^{13}C_D)[C]_B f_{B,BM} + (\delta^{13}C'_C - \delta^{13}C_D)[C]_C f_{C,BM} = 0$$

$$(\delta^{15}N'_A - \delta^{15}N_D)[N]_A f_{A,BM} + (\delta^{15}N'_B - \delta^{15}N_D)[N]_B f_{B,BM} + (\delta^{15}N'_C - \delta^{15}N_D)[N]_C f_{C,BM} = 0$$

$$f_{A,BM} + f_{B,BM} + f_{C,BM} = 1$$

where $f_{A,B}$, $f_{B,B}$, and $f_{C,B}$ represent the fractional contributions of assimilated biomass (*BM*) of the potential food sources *A*, *B*, and *C*, within the mixture *D*. Carbon concentrations in the food sources are represented in the model by $[C]_A$, $[C]_B$, $[C]_C$; nitrogen concentrations are represented as $[N]_A$, $[N]_B$, $[N]_C$. $\delta^{13}C_{POM}$ will be determined by averaging the values obtained from previously published studies. When assessing the proportion of food source contributions, step-wise enrichment of carbon and nitrogen stable isotopes (*ie*. fractionation) requires consideration (Dubois *et al*. 2007). Trophic fractionation is represented by the prime (') symbol within the above model. Generally, average fractionation of each species increases consistently and predictably to each trophic level. Measuring fractionation of $\delta^{13}C$ can define basal sources of productivity and primary producers; measuring fractionation of $\delta^{15}N$ can describe trophic level of species under examination (Gannes *et al.* 1997; Sweeting *et al.* 2005). Many studies have determined that the average ^{13}C enrichment is equal to 1 ‰ and the average ^{15}N enrichment is equal to 3 ‰ per trophic level (Peterson & Fry 1987; Deegan & Garrett 1997; Kharlamenko *et al.* 2001; Riera *et al.* 2002). Therefore, these values were used for the 3-source isotopic mixing model. Carbon and nitrogen concentration and contribution to consumer (*M. edulis* and *C. inflata*) diets were calculated with IsoSource version 1. 3. 1 (Phillips & Gregg 2003), which calculates and compiles all possible feasible solutions that could explain isotopic signatures in each sample. IsoSource analyses 0 to 100 % possible combinations of potential contributions of each food source in minimal increments (1 % in this study) and equating within 0.1 % of the average isotopic signatures of the consumers (Phillips & Gregg 2003; Benstead *et al*. 2006; Quan *et al*. 2007). The user specified tolerance values were set to 2.6 ‰ for sablefish, 2.5 ‰ for blue mussels, 1.9 ‰ for sea urchins, 0.12 ‰ for Pacific scallops, 2.2 ‰ for broadbase tunicates, ‰ for brooding transparent tunicates, and 1.9 ‰ for hairy tunicates. Trophic fractionation was not accounted for within the source data prior to inputting to software for analysis due to the relatively small trophic fractionation of C isotopes and the lack of

information regarding the number of trophic levels between primary (*ie*. basal) sources and consumer sources (Benstead *et al*. 2006).

3.3.6 Statistical Analysis

Prior to statistical analysis, raw data were tested for normality using Kolmogorov-Smirnov test, and homogeneity of variance using Levene's test. In each test, 95% significance level was adhered to. A one-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test for multiple comparisons, were performed to quantitatively estimate any interspecific and intraspecific differences within and among each sampling group, as well as any temporal variation between sampling dates for IMTA samples (Zar 1999). All statistical analyses were performed using software SPSS version 17.0 for Windows (SPSS 17.0, Chicago, IL) except for Rayleigh's *Z* and *P* tests for uniformity of angular distributions as well as the Watson-Williams multisample *F* test for differences between mean angles of direction for different monthly sampling transitions, which were performed using Oriana 3 (Rockware, Inc., Golden, Colorado, USA).

Data in the present study represent nearly a full year of sampling hence isotopic signatures were converted into degrees or angles within a circular distribution for one year. These degrees represent directional changes in food web niche space over time (expressed in magnitude and direction). $\delta^{13}C$ and $\delta^{15}N$ signatures were corrected for circular analysis. Rayleigh's test (Zar 1999) was employed in order to test the null hypothesis that the isotopic signatures among samples do not vary in time. This test for randomness is based on the von Mises distribution, which is the angular equivalent to the normal distribution, in order to test randomness (Batschelet 1981; Jelinski *et al*. 2002). This statistical method graphically presents data as arrow diagrams, where isotopic values are distributed along the circumference of a circle, which represents time (Zar 1999). The arrow represents a sample group; the direction of the arrow represents the direction of change within trophic niche space; and the length of the arrow represents how far the sample group has moved within trophic niche space (Schmidt *et al*. 2009). Arrow diagrams were created using the Oriana 3 software package.

3.4 Results

3.4.1 Food Web Reconstruction and Food Source Partitioning Within IMTA Site

June and July 2009 were the only months whereby a complete set of samples was obtained. Figure 3.3 is a dual isotope plot for these months. It features the relative trophic relationships, or isotopic niche space, between the cultivated (*ie*. intended) aquaculture extractives *Anoplopoma fimbria* (δ^{13}C: -20.90 ± 0.8 ‰, δ^{15}N: 13.22 ± 0.4 ‰), *Strongylocentrotus franciscanus* (δ^{13}C: -19.71 ± 0.8 ‰, δ^{15}N: 9.57 ± 0.7 ‰), *Mytilus edulis* (δ^{13}C: -18.97 ± 0.2 ‰, δ^{15}N: 8.88 ± 0.8 ‰), *Patinopectin yessoensis* (δ^{13}C: -22.16 ± 1.0 ‰, δ^{15}N: 8.19 ± 0.2 ‰), and *Saccharina latissima* (δ^{13}C: -17.26 ± 2.1 ‰, δ^{15}N: 5.11 ± 1.8 ‰), the aquaculture-derived waste effluent of uneaten fish feed (δ^{13}C: -21.62 ± 0.5 ‰, δ^{15}N: 10.48 ± 0.5 ‰) and *A. fimbria* excretion (δ^{13}C: -22.11 ± 0.7 ‰, δ^{15}N: 10.66 ± 1.1 ‰), and the adjacent biofouling species *Cnemidocarpa finmarkeinsis* (δ^{13}C: -19.40 ± 0.6 ‰, δ^{15}N: 9.10 ± 0.4 ‰)**,** *Corella inflata* (δ^{13}C: -18.28 ± 1.4 ‰, δ^{15}N: 5.52 ± 1.8 ‰)**,** *Boltenia villosa* (δ^{13}C: -19.15 ± 0.5 ‰, δ^{15}N: 8.69 ± 0.4 ‰)**,** *Parastichopus californicus* (δ^{13}C: -17.99 ± 0.3 ‰, δ^{15}N: 8.61 ± 0.7 ‰)**,** and *Rhodophyte* sp. (δ^{13}C: -29.07 ± 2.3 ‰, δ^{15}N: 5.78 ± 1.0 ‰).

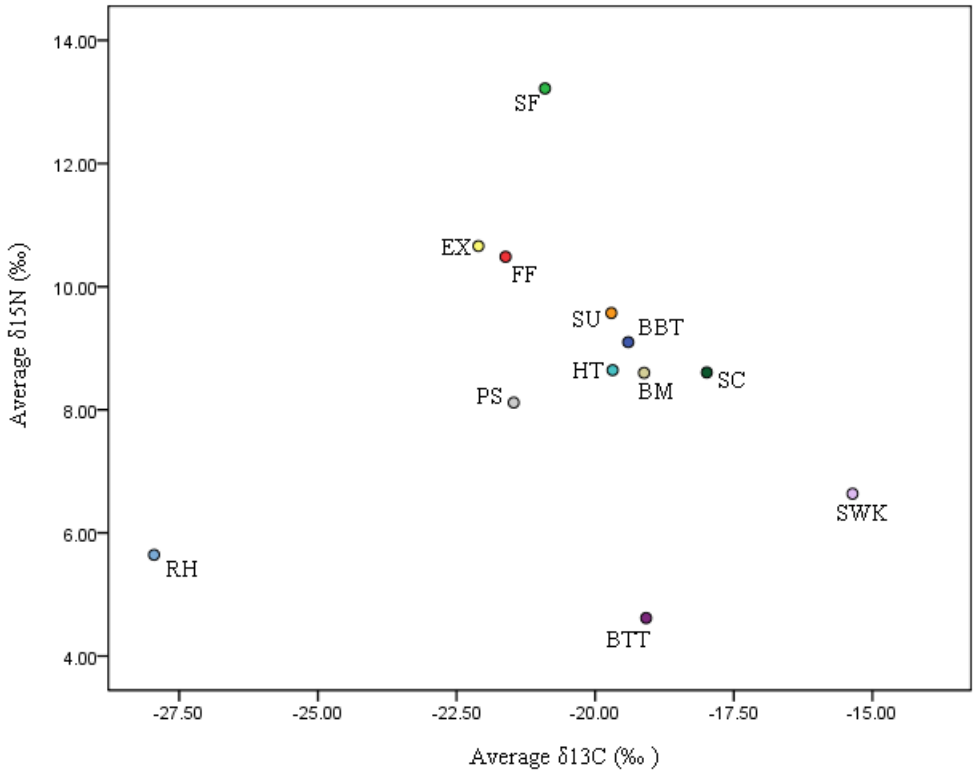


Figure 3.3 Dual isotope plot of stable carbon and nitrogen averages taken from sample groups obtained in June and July 2009 from the IMTA project site. Sample abbreviations are as follows: SF, sablefish (*Anoplopoma fimbria*), EX, fish excretion, FF, fish feed, SU, sea urchin (*Stronglocentrotus franciscanus*), BBT, broadbase tunicate (*Cnemidocarpa finmarkeinsis*), HT, hairy tunicate (*Boltenia villosa*), BM, blue mussel (*Mytilus edulis*), SC, sea cucumber (*Parastichopus californicus*), PS, Pacific scallop (*Patinopectin yessoensis*), SWK, sugar-wrack kelp (*Saccharina latissima*), BTT, brooding transparent tunicate (*Corella inflata*), and RH, *Rhodophyte* sp.

The trend depicted in Figure 3.3 shows spatial relationships of isotopic values that generally match the spatial and physical placement of sample organisms within the IMTA project site. As predicted, average isotopic values for sablefish were more enriched in both $\delta^{13}C$ and $\delta^{15}N$ than its source fish feed. Also predicted were the similar values for

both fish feed and sablefish excretion. With the exception of the average isotopic value for brooding transparent tunicates, clustered together are the sample organisms that were retrieved within nearby shellfish nets (e.g. blue mussel, sea urchin, sea cucumber, broadbase tunicate, hairy tunicate, and Pacific scallop). *Rhodophyte* sp. and sugar-wrack kelp samples were retrieved from the far end of the IMTA project site and had the smallest levels of nitrogen enrichment.

The one-way ANOVA of summer 2009 $\delta^{13}C$ signatures performed between the thirteen sampling groups determined that there is significant difference between mean signatures (df = 12, F = 28.018, MS = 47.292, p = 0.000). A significant difference between summer 2009 means was also observed for $\delta^{15}N$ signatures between all sampling groups (df = 12, F = 18.132, MS = 29.998, p = 0.00). This test was performed in order to quantitatively estimate any differences between each isotopic signature for each sample.

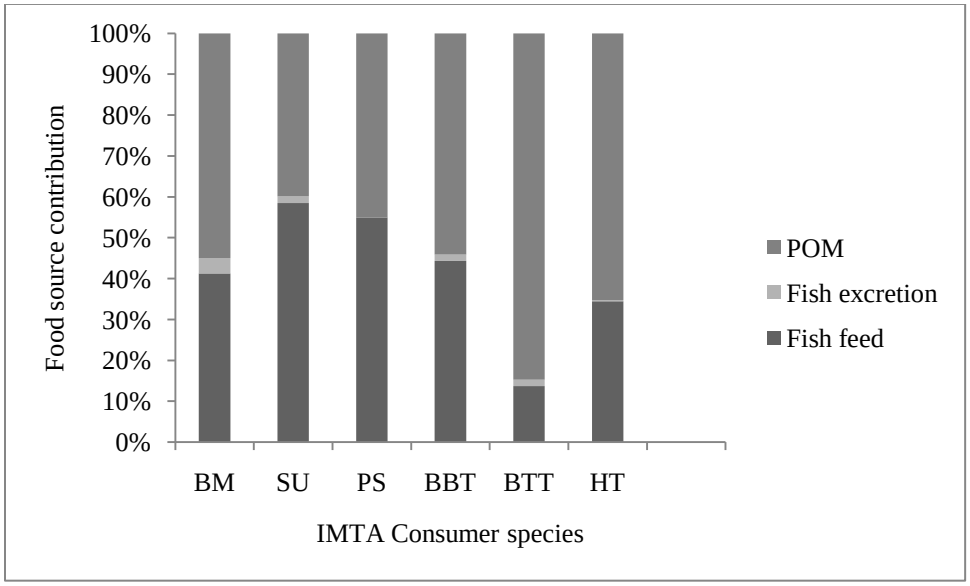


Figure 3.4 Histogram of interspecific variability of average relative contributions (%) of food sources for each IMTA sampled consumer species as estimated by 3-sources mixing model software IsoSource version 1.3.1. Food sources include particulate organic matter (POM) which represent a hypothetical example taken from the literature, fish excretion and fish feed. Abbreviations of consumer species include: BM, blue mussel (*Mytilus edulis*), SU, sea urchin (*Strongylocentrotus franciscanus*), PS, Pacific scallop

(*Patinopectin yessoensis*), BBT, broadbase tunicate (*Cnemidocarpa finmarkeinsis*), BTT, brooding transparent tunicate (*Corella inflata*), and HT, hairy tunicate (*Boltenia villosa*).

3.4.2 Temporal Variability of IMTA Site Extractives and Biofouling Organisms

The mean isotopic carbon and nitrogen values for each IMTA site sample group are presented for each sampling period in Table 3.2. A one-way ANOVA did not reveal a significant difference between sampling periods for $\delta^{13}C$ values for all samples (df = 8, MS = 3.703, F = 0.630, p = 0.750). Similar results were measured for all $\delta^{15}N$ values of samples, revealing no significant difference between sampling periods (df = 8, MS = 3.062, F = 0.581, p = 0.790).

Table 3.2 Carbon and nitrogen isotopic signatures of organic matter samples averaged by sampling date. n = number of observations; $\delta^{13}C$ = carbon isotopic values (‰); $\delta^{15}N$ = nitrogen isotopic values (‰); ± s.e. = standard errors for the means).

Sample	**Sampling dates**	**$\delta^{13}C$ ± s.e.**	***n***	**$\delta^{15}N$ ± s.e.**	***n***
Anoplopoma fimbria (sablefish)	July 2009	-20.90 ± 0.8	4	13.22 ± 0.4	4
	August 2009	-22.84 ± 0.7	2	12.02 ± 0.8	2
	October 2009	-22.95 ± 0.7	3	11.70 ± 0.7	3
	November 2009	-23.12 ± 0.2	3	11.99 ± 0.6	3
	March 2010	-22.62 ± 0.4	2	10.36 ± 1.2	2
	April 2010	-22.73 ± 0.2	3	11.16 ± 0.2	3
Mytilus edulis (blue mussel)	June 2009	-18.91 ± 0.2	5	8.99 ± 0.9	5
	July 2009	-19.12 ± 0.3	2	8.60 ± 0.5	2
	August 2009	-17.14 ± 0.1	2	8.03 ± 0.6	2
	October 2009	-17.41 ± 0.6	5	8.76 ± 0.2	5
	November 2009	-17.98 ± 0.2	3	8.64 ± 0.3	3

	January 2010	-18.14 ± 0.3	3	8.43 ± 0.5	3
	February 2010	-17.85 ± 0.6	3	9.35 ± 0.2	3
	March 2010	-18.76 ± 0.1	3	9.10 ± 0.2	3
	April 2010	-20.35 ± 0.2	3	8.00 ± 0.2	3
Strongylocentrotus franciscanus (sea urchin)	July 2009	-19.71 ± 0.8	3	9.57 ± 0.7	3
	August 2009	-18.71 ± 2.5	2	10.29 ± 0.5	2
	October 2009	-17.52 ± 1.4	5	9.10 ± 0.6	5
	November 2009	-17.00 ± 1.0	3	8.60 ± 0.3	3
	January 2010	-16.95 ± 0.6	4	9.15 ± 0.5	4
	February 2010	-17.19 ± 0.5	2	9.25 ± 0.2	2
	March 2010	-16.66 ± 0.4	2	9.70 ± 0.5	2
	April 2010	-16.02 ± 0.2	3	9.31 ± 0.1	3
Patinopecten yessoensis (Pacific scallop)	June 2009	-22.69 ± 0.4	5	8.28 ± 0.2	5
	July 2009	-20.83 ± 0.2	2	7.99 ± 0.2	2
	August 2009	-21.00 ± 0.2	3	8.39 ± 0.03	3
	October 2009	-19.50 ± 0.4	2	8.42 ± 0.3	2
	November 2009	-20.52 ± 0.2	3	8.47 ± 0.2	3
	January 2010	-18.73 ± 0.6	3	9.66 ± 0.1	3
	February 2010	-19.46 ± 0.4	3	9.55 ± 0.2	3
	March 2010	-19.89 ± 0.3	3	8.20 ± 0.5	3
	April 2010	-20.45 ± 0.4	3	8.36 ± 0.3	3
Cnemidocarpa finmarkeinsis	July 2009	-19.40 ± 0.6	2	9.10 ± 0.4	2

(broadbase tunicate)	August 2009	-18.11 ± 0.3	2	8.98 ± 0.1	2
	October 2009	-18.77 ± 0.6	5	9.45 ± 0.1	5
	November 2009	-18.85 ± 0.5	3	9.17 ± 0.2	3
	January 2010	-18.96 ± 0.8	3	8.54 ± 0.1	3
	February 2010	-18.33 ± 2.3	3	10.71 ± 0.1	3
	March 2010	-16.27 ± 3.5	2	8.82 ± 1.8	2
	April 2010	-20.44 ± 0.5	3	9.68 ± 0.5	3
Corella inflata	June 2009	-17.88 ± 1.5	5	5.97 ± 2.1	5
(brooding transparent tunicate)	July 2009	-19.08 ± 0.7	2	4.62 ± 0.8	2
	August 2009	-17.45 ± 1.2	2	5.15 ± 1.2	2
	October 2009	-16.21 ± 4.1	5	4.43 ± 3.6	5
	November 2009	-18.97 ± 1.2	3	4.56 ± 2.4	3
	January 2010	-20.13 ± 0.6	2	5.02 ± 0.6	2
	February 2010	-22.05 ± 0.3	2	6.81 ± 0.7	2
	March 2010	-19.50 ± 2.9	3	7.09 ± 0.6	3
	April 2010	-21.94 ± 0.2	3	5.13 ± 1.2	3
Boltenia villosa (hairy tunicate)	June 2009	-18.94 ± 0.4	5	8.71 ± 0.4	5
	July 2009	-19.68 ± 0.3	2	8.64 ± 0.4	2
	August 2009	-17.58 ± 0.1	2	8.20 ± 0.2	2
	October 2009	-18.34 ± 0.7	5	8.73 ± 0.7	5
	November 2009	-18.86 ± 0.1	3	8.59 ± 0.3	3
	January 2010	-18.12 ± 0.5	3	8.98 ± 0.1	3

	February 2010	-18.12 ± 1.2	3	10.55 ± 0.5	3
	March 2010	-19.63 ± 0.02	3	9.54 ± 0.6	3
	April 2010	-19.70 ± 0.6	3	8.74 ± 0.8	3
Parastichopus californicus (sea cucumber)	July 2009	-17.99 ± 0.3	2	8.61 ± 0.7	2
Saccharina latissima (sugar-wrack kelp)	June 2009	-18.41 ± 0.8	5	4.20 ± 1.5	5
	July 2009	-15.36 ± 2.5	3	6.64 ± 0.8	3
	August 2009	-20.55	1	1.80	1
	October 2009	-18.68 ± 3.2	2	4.89 ± 0.7	2
	March 2010	-18.17 ± 0.9	3	5.68 ± 1.5	3
Rhodophyte sp.	June 2009	-29.81 ± 0.8	3	5.87 ± 1.4	3
	July 2009	-27.96 ± 4.0	2	5.64 ± 0.2	2
	August 2009	-21.52 ± 3.1	2	4.65 ± 2.3	2
Fish feed	January 2010	-21.62 ± 0.5	3	10.48 ± 0.5	3
Sablefish excretion	July 2009	-22.11 ± 0.7	4	10.66 ± 1.1	4
	August 2009	-21.30 ± 0.2	2	12.15 ± 0.3	2
	October 2009	-22.82 ± 0.4	3	10.82 ± 0.5	3
	November 2009	-20.58 ± 0.8	3	12.18 ± 0.6	3
	January 2010	-22.40	1	n/d	
	March 2010	-20.57 ± 0.3	2	13.10 ± 0.3	2
	April 2010	-20.86 ± 0.2	3	12.18 ± 0.8	3

Monthly $\delta^{13}C$ and $\delta^{15}N$ mean values for all sample groups from June 2009 to April 2010 are presented in Figures 3.5 and 3.6, respectively, for the IMTA site. Sugar-

wrack kelp (*S. latissima*) and *Rhodophyte* sp. exhibited the most pronounced $\delta^{13}C$ variations in the summer 2009. This is followed by the broadbase tunicate (*C. finmarkeinsis*) which depleted drastically between March and April 2010. The remaining tunicate species (*C. inflata*, and *B. villosa*) and the sea urchin (*S. franciscanus*) generally follow the same pattern and with similar average values. The Pacific scallop (*P. yessoensis*) generated a gradual enrichment between the first sampling date and the final. Monthly values for sablefish (*A. fimbria*) remain consistently and slightly more depleted than fish feed and fish excretion averages. The majority of sampling groups measured more depleted in $\delta^{15}N$ than fish feed, suggesting that fish feed does not serve as a single or primary source of food for most samples. There must be one or more lower trophic food sources unaccounted for within the diets of sampled consumers. As expected, both $\delta^{15}N$ sablefish excretion and sablefish values averaged higher (*ie*. more enriched) than fish feed. With the exception these, as well as of *Rhodophyte* sp. (there were not enough samples), sugar-wrack kelp, and the brooding transparent tunicate, all other sample groups displayed an overlapping monthly trend, including a slight enrichment spike in February 2010. Marine POM averages from the literature were intentionally left out of the temporal study in order to keep the level of estimation to a minimum.

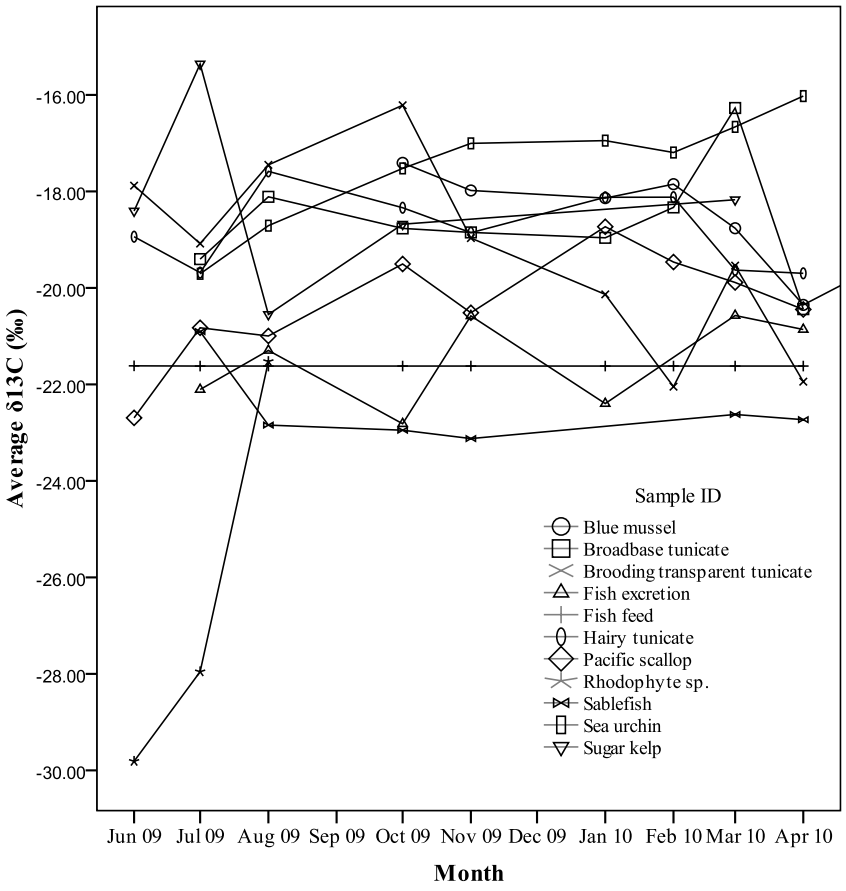


Figure 3.5 Average $\delta^{13}C$ values (‰) for sample groups collected at IMTA site over duration of sampling period (June 2009 to April 2010).

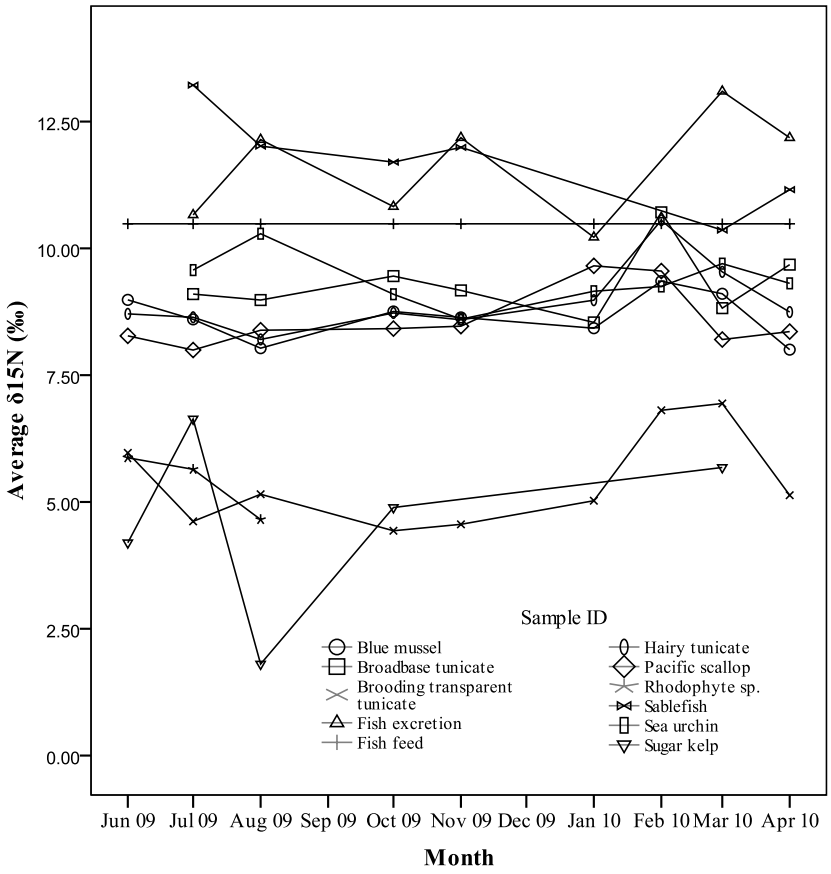


Figure 3.6 Average $\delta^{15}N$ values (‰) for sample groups collected at IMTA site over duration of sampling period (June 2009 to April 2010).

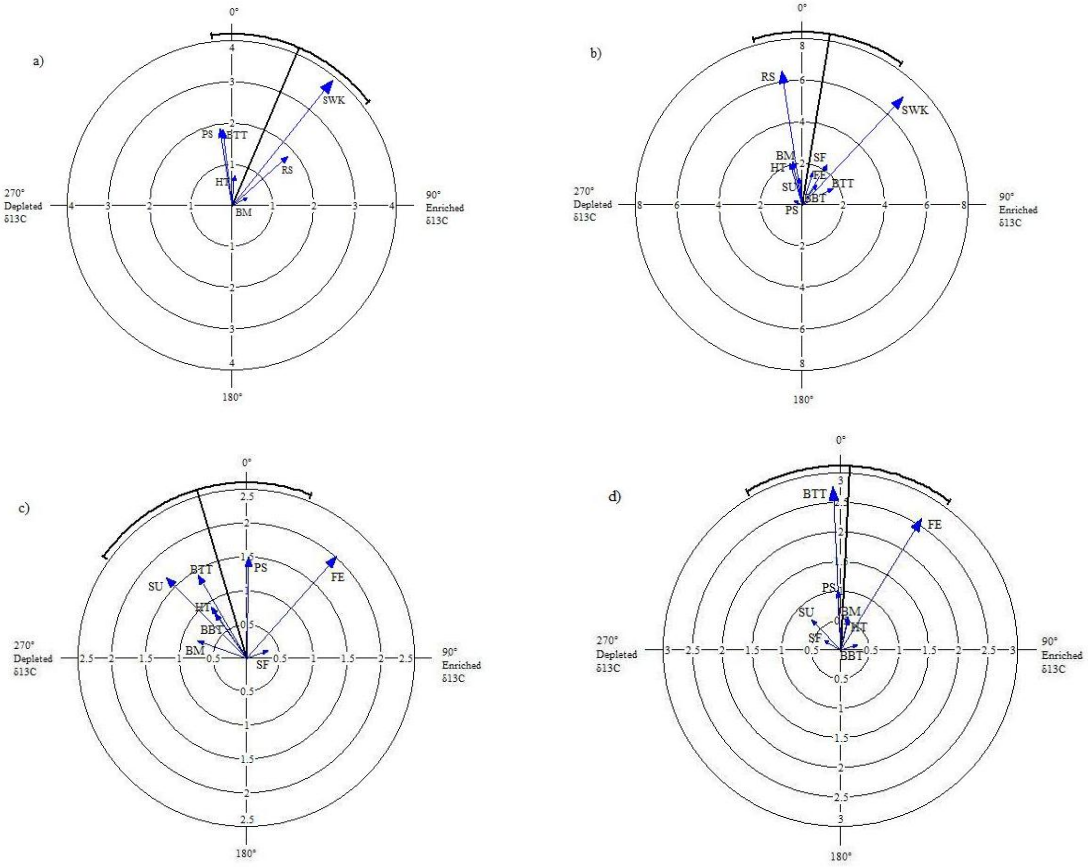
Directional change (magnitude and angle) of each sampling group through each of the eight consecutive sampling months was quantified and presented in Table 3.3 and Figure 3.7 (*a*—*h*). Nearly all sampling month transitions showed significant directionality in sample groups' isotopic signature shifts within trophic niche space (Rayleigh's test, $P < 0.05$; Table 3.4; Figure 3.7*a*—*e*, *g* & *h*). Between sample months January to February 2010, there was no significant directionality as measured by Rayleigh's test $P = 0.06$; Table 3.4; Figure 3.7*f*). Rayleigh's test is used to measure the distribution of mean angles of direction and whether they depart from uniformity (Schmidt *et al*. 2009).

Table 3.3 Directional change (magnitude and θ (in degrees)) of each sample group over each sampling period within the IMTA project site.

Sample	June 09--July 09		July 09--August 09		August 09--October 09		October 09--November 09	
	Magnitude	θ (°)	Magnitude	θ (°)	Magnitude	θ (°)	Magnitude	θ (°)
A. fimbria (sablefish)	ND	ND	2.28	31.7	0.34	71.0	0.34	300.4
M. edulis (blue mussel)	0.43	61.1	2.06	343.9	0.78	290.3	0.58	11.9
S. franciscanus (sea urchin)	ND	ND	1.23	35.8	1.68	315.0	0.72	316.1
P. yessoensis (Pacific scallop)	1.88	351.4	0.43	293.6	1.5	1.1	1.02	357.2
C. finmarkeinsis (broadbase tunicate)	ND	ND	1.3	354.7	0.81	324.5	0.29	74.1
C. inflata (brooding transparent tunicate)	1.8	48.4	1.71	18.0	1.43	329.9	2.76	357.3
B. villosa (hairy tunicate)	0.74	5.4	2.15	348.2	0.93	325.1	0.54	15.1
S. latissima (sugar-wrack kelp)	3.91	38.7	7.1	43.0	ND	ND	ND	ND
Rhodophlye sp.	1.86	352.9	6.52	351.3	ND	ND	ND	ND
Fish excretion	ND	ND	1.7	61.5	2.02	41.2	2.62	31.3

Sample	November 09--January 10		January 10--February 10		February 10--March 10		March 10--April 10	
	Magnitude	θ (°)	Magnitude	θ (°)	Magnitude	θ (°)	Magnitude	θ (°)
A. fimbria (sablefish)	ND	ND	ND	ND	ND	ND	0.81	277.8
M. edulis (blue mussel)	0.26	52.7	1.35	42.9	0.94	15.4	1.93	34.7
S. franciscanus (sea urchin)	0.55	84.8	0.26	337.4	0.7	40.3	0.75	328.6
P. yessoensis (Pacific scallop)	21.5	33.6	0.74	8.6	1.48	72.3	0.58	344.6
C. finmarkeinsis (broadbase tunicate)	0.64	80.1	2.26	73.8	2.8	317.5	4.26	348.3
C. inflata (brooding transparent tunicate)	1.25	338.4	2.63	317.0	2.51	3.0	3.01	37.0
B. villosa (hairy tunicate)	0.84	27.8	1.57	90.0	1.82	0.8	0.8	85.0
S. latissima (sugar-wrack kelp)	ND	ND	ND	ND	ND	ND	ND	ND
Rhodophlye sp.	ND	ND	ND	ND	ND	ND	ND	ND
Fish excretion	2.68	47.1	ND	ND	ND	ND	0.97	72.5

Directional food web changes (Figure 3.7a—h) are depicted in each diagram within $\delta^{13}C$—$\delta^{15}N$ space between each month: a) June ‘09 to July ‘09, b) July ‘09 to August ‘09, c) August ‘09 to October ’09, d) October ’09 to November ’09, e) November ’09 to January ’10, f) January ’10 to February ’10, g) February ’10 to March ’10, and h) March ’10 to April ’10. Each arrow represents a different sample group, the direction of the arrow represents the isotopic change with which the sample group moved from one month to another, and the length of the arrow represents the magnitude of change (‰) in trophic niche space. The concentric circles depict magnitude of change increasing outwards corresponding to increasing trophic position from one sample session to the next. The bold line dissecting the circles represents the mean vector of change (μ), and the bold outer arc segment represents the 95% confidence interval around the mean vector of change. All sampling date transitions, except for the August to October 2009 shift, displayed mean isotopic transitions to more enriched $\delta^{13}C$ values (Figure 3.7*a*, *b*, *d*—*h*; Table 3.4). The monthly transition June to July 2009 measured a mean vector (μ) of 22.9°, July to August 2009 measured 9.3°, October to November 2009 measured 2.7°, November 2009 to January 2010 measured 44.8°, January to February 2010 measured 25.3°, February to March 2010 measured 21.3°, and March to April 2010 measured 12.9°. As for the sampling month transition between August and October 2010, the mean vector was calculated to be 343.4° which is indicative of a shift to more negative $\delta^{13}C$ values (Figure 3.7*c*; Table 3.4). Here fish excretion values displayed the most notable shift among all groups sampled between these two months. The shifts exhibited in both Figure 3.7*f* and *g* support the qualitative observational $\delta^{15}N$ spike in Figure 3.6 at around the same time frame. These shifts appear within the region of increased trophic position and at a relatively greater magnitude of change (at the 0° axis). Similarly, the large magnitude in shift observed in Figure 3.5 by sugar-wrack kelp is quantitatively represented in Figure 3.7*a* and *b*.


a)
0°
90°
Enriched
δ13C
180°
270°
Depleted
δ13C
SWK
PS
STT
RS
HT
BM
b)
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SWK
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HT
SU
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BBT
PS
c)
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BBT

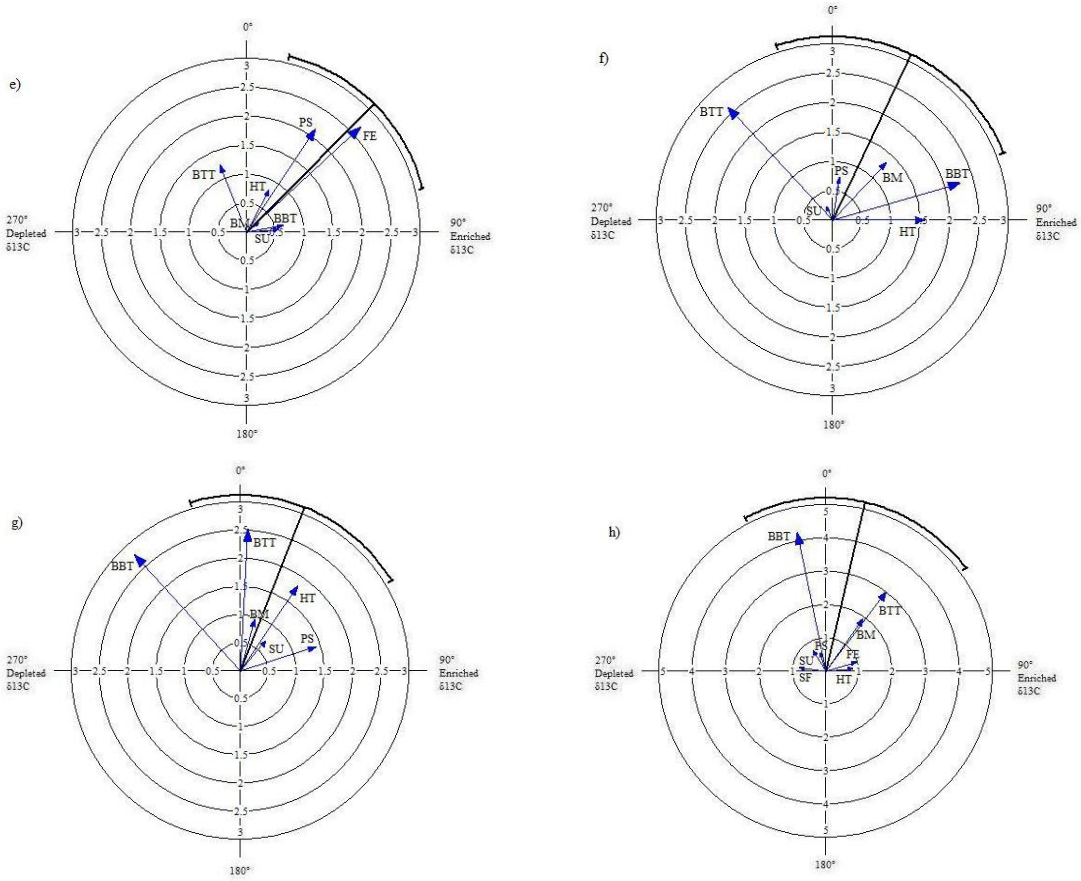

Figure 3.7 Arrow diagrams for angle (θ) and magnitude (arrow length) of change for each IMTA sample group between time periods. The data label abbreviations are as follows: SF, sablefish (*Anoplopoma fimbria*), BM, blue mussel (*Mytilus edulis*), SU, sea urchin (*Strongylocentrotus franciscanus*), PS, Pacific scallop (*Patinopecten yessoensis*), BBT, broadbase tunicate (*Cnemidocarpa finmarkiensis*), BTT, brooding transparent tunicate (*Corella inflata*), HT, hairy tunicate (*Boltenia villosa*), SWK, sugar-wrack kelp (*Saccharina latissima*), RS, Rhodophyte sp., FE, fish excretion.

The multisample Watson-Williams F-test was performed on all eight sampling transition periods and revealed that all eight calculated angles of change are from populations with different mean angles ($F_{7,\ 51} = 1.243$, $P = 0.297$). In other words, the overall direction of change in food web space among all sampled groups has not been consistent over the course of this study (Schmidt *et al*. 2007).

Table 3.4 Circular statistical values for a number of variables describing sample groups within the IMTA project site over each consecutive sampling period. Rayleigh's test values marked with an asterisk (*) are significant at the α = 0.05 level, meaning the distribution of angular variance departs from uniformity.

Variable	**Jun 09—Jul 09**	**Jul 09—Aug 09**	**Aug 09—Oct 09**	**Oct 09—Nov 09**
Number of observations	6	10	8	8
Mean vector (μ)	22.9	9.3	343.4	2.7
Length of mean vector (r)	0.89	0.83	0.73	0.79
Circular standard deviation	28.0	35.5	45.0	39.5
Rayleigh's test (Z statistic)	4.73	6.81	4.31	4.97
Rayleigh's test (P)	0.004*	2.48E-0.4*	0.009*	0.003*

Variable	**Nov 09—Jan 10**	**Jan 10—Feb 10**	**Feb 10—Mar 10**	**Mar 10—Apr 10**
Number of observations	7	6	6	8
Mean vector (μ)	44.8	25.3	21.3	12.9
Length of mean vector (r)	0.84	0.67	0.82	0.63
Circular standard deviation	33.4	50.9	35.8	20.8
Rayleigh's test (Z statistic)	4.99	2.72	4.06	3.21
Rayleigh's test (P)	0.003*	0.06	0.01*	0.035*

3.5 Discussion

Investigations into biomitigation and assimilation of aquaculture-derived waste by adjacent marine communities are becoming more common (Chopin *et al*. 2001; Reid *et al*. 2009; Troell *et al*. 2009). Concerns involving the influence of nearby biofouling organisms include interspecific competition with intended organic and inorganic extractives may affect calculations in carrying capacity and monitoring. Certainly,

seasonal fluctuations in aquaculture waste as a subsidy may also effect rate of biomitigation and assimilation. In an integrated multi-trophic aquaculture project located on the northwest coast of Vancouver Island, stable $\delta^{13}C$ and $\delta^{15}N$ analysis has provided preliminary results that could lead to better understanding of trophic linkages that exist between farmed sablefish, intended extractive filter feeders and algae, and biofouling epibionts over the course of nearly a year.

Qualitative and quantitative results from both the dual isotope plot (Figure 3.3) and the three-source mixing model (Figure 3.4) corroborate variable proportions of aquaculture-derived waste relied upon for food by both intended (extractives: sablefish, blue mussels, Pacific scallops, sea urchins) and unintended (biofouling organisms: broadbase tunicates, brooding transparent tunicates, hairy tunicates, sea cucumber) organisms sampled within the vicinity of the IMTA project site. This exercise has also permitted a better understanding into potential subsidies supporting unintended, or biofouling, organisms within the IMTA project vicinity. It is likely that aquaculture waste is incorporated into a mixed diet of these biofouling organisms and, when comparing IsoSource output to the qualitative dual isotope and isotope versus time plots, do not necessarily exhibit considerable niche overlap or competition for resources. In fact, one common biofouling epibiont sampled, the brooding transparent tunicate *C. inflata*, exhibited very little reliance on aquacultural effluent, despite being at high density within the vicinity of other sample organisms and the IMTA project site. Similar results were found in a study by Dubois *et al*. (2007) analyzing food partitioning of another ascidian species, *Ascidiella aspersa*. They gathered isotopic data on a number of epibionts near cultivated oysters, and evidence from four-source mixing model (also via IsoSource) revealed depletion and low variability in $\delta^{15}N$ values, as well little overall isotopic overlap of *A. aspersa* relative to other groups sampled. Dubois *et al*. (2007) suggest that hydrodynamic conditions including high velocity ebb and flow tides create turbidity in the water column that resuspends sediment and increases concentration of inorganic particles, at which time, potentially interferes with feeding activity. They proposed that *A. aspersa* only feed at high tides when such activities stabilize, and thereby confirming their results indicating low trophic plasticity in the species.

The dual isotope plot revealed a possible trend relating the cascading order of sample groups' isotopic signatures with a direct spatial order within the IMTA site inside which they were sampled. Circular tidal currents from the open ocean make first contact with the sablefish cages which then carry aquaculture-generated particulate matter towards a series of lantern nets whereby all biofouling organisms (eg. tunicates) and most intended extractives (eg. blue mussels, sea urchins) are recruited. Pacific scallops are reared in nets slightly down current followed by lines of sugar-wrack kelp several meters away at the end of the project site. Two sample species, *Rhodophyte* sp. and brooding transparent tunicate revealed $\delta^{13}C$ and $\delta^{15}N$ values outside of the cascading trend along the dual isotope plot.

$\delta^{15}N$ values for each sampling group plotted against time revealed three main trophic groups. Sablefish exhibited the most enriched values overall, followed by levels detected for fish feed. The second trophic group included all invertebrate sample species except for brooding transparent tunicates, which measured depleted values more similar to sugar-wrack kelp, which comprises the third trophic group. This is also confirmed by the IsoSource output that has revealed each of these trophic groupings to exhibit separate levels of food source reliability. In the first group, sablefish demonstrated to rely solely on fish feed; in the second grouping, all invertebrate sample groups (except for brooding transparent tunicates), rely on a mixture of both aquaculture-generated waste and other primary sources; brooding transparent tunicate, despite their close proximity to the IMTA sampling site within which they were collected, seem to exhibit negligible reliance on aquaculture-generated waste for diet. Monthly direct isotopic measurements of POM, sedimentary and benthic organic matter could potentially better delineate each trophic group sampled at this site.

Unlike related studies analyzing temporal changes in isotopic signatures within marine ecosystems (see Simenstad & Wissmar 1985; Goering *et al*. 1990; Kang *et al*. 1999; Vizzini & Mazzola 2002; Vizzini & Mazzola 2003), there was no clear detection of ^{13}C isotopic enrichment in summer months and depletion in winter months among sampling groups in the present study. Since seasonal variation in marine ecosystems

tends to be related to environmental factors, such as temperature, freshwater and/or terrestrial inputs, water movements, carbon and nitrogen food source availability, and irradiance levels, perhaps the lack of notable isotopic seasonal variation may not be detectable as these environmental factors may not vary drastically (see Goering *et al*. 1990 and references therein). It is likely, however, the constant supply of IMTA feed particles are dampening any seasonal fluxes. Further investigation in to the seasonal variability of these environmental factors would greatly compliment this study.

A red tide event was observed at the IMTA project site in late August 2009, and various abrupt changes were noted qualitatively in Figures 3.5 and 3.6, and qualitatively in Figure 3.7*b*. Most notable were the significant depletion of $\delta^{13}C$ and $\delta^{15}N$ values in sugar-wrack kelp and enrichment in $\delta^{13}C$ values observed in *rhodophyte* sp. These observations were further corroborated by the long arrows of magnitude demonstrated by these particular sample groups. A study by Minagawa & Wada (1984) revealed abnormally lower $\delta^{15}N$ values for zooplankton and fish sampled during red tide, as compared to related organisms from other ecosystems. They suggested that primary producers exhibiting lower $\delta^{15}N$ values depend primarily on atmospheric nitrogen fixation. Since $\delta^{13}C$ and $\delta^{15}N$ values of each sample organism was relatively mixed, different sources of carbon and nitrogen must also be mixed (Minagawa & Wada 1986; Vizzini & Mazzola 2003). Furthermore, the $\delta^{15}N$ values of most sample consumers measured in the present study changed very little before, during, and after the red tide event suggesting that their diets rely very little on atmospheric nitrogen-fixing primary sources.

Circular statistics revealed temporal variation within sampling groups' trophic niches but did not indicate any particular or significant overall directional change in marine food web space. Other food web studies that have employed quantitative statistical methods to trace food webs over time have generally found very little directional changes in food web space over much larger temporal spans, until a major event (*eg*. non-native species introduction) directly impacts the environment under study (Schmidt *et al*. 2007; Schmidt *et al*. 2009; Vander Zanden *et al*. 2006). In the present

study, there were no anthropomorphic-driven biological events that were introduced to the system within the temporal span of sampling periods.

Other ecological studies employing stable isotope data have used circular statistical metrics in order to assess the effects of species introduction on an ecosystem over long time scales (Schmidt *et al*. 2007; Schmidt *et al*. 2009). Schmidt *et al*. (2007) employed circular statistical methods for hypothesis testing on both spatial and temporal directional food web differences within two separate ecological communities. Schmidt *et al*. (2009) later examined the effects of the introduction of non-native fishes such as the sea lamprey *Petromyzon marinus*, the rainbow smelt *Osmerus mordax*, and the alewife *Alosa pseudoharengus*, to the niche partitioning with native species within the Lake Superior fish community. The arrow diagrams revealed negligible shifts in trophic niche space for native species after the introduction of non-native fishes and significant shifts for non-native species *P. marinus* and *O. mordax*, supporting the ability of Lake Superior to accommodate both native and non-native fish communities without drastic and detrimental effects.

Dekar *et al*. (2009) conducted a seasonal investigation that used circular statistical metrics coupled with mixing models in order to measure the relative contribution of autochthonous and allochthonous energy sources as well as temporal and spatial interactions among stable isotope data collected seasonally from stream systems in Arkansas, US. By incorporating both circular statistics with mixing models, this study better attempted to answer questions regarding both directional significance (by testing hypotheses about changes along temporal and spatial gradients) and magnitude of change among sampled consumers, than by single-faceted analysis alone. This study demonstrated the utility of employing various analytical tools in order to investigate variation in consumer-resource dynamics affecting food web stability that could affect our understanding of resource management and conservation. At present, there are no studies that have employed a similar multi-faceted approach to understanding temporal and spatial food web dynamics within an integrated multi-trophic aquaculture (IMTA) site.

Out of the three food sources identified in this study, only two of them were measured directly at this site. Proper understanding of baseline isotopic signatures of food resources can greatly assist in understanding trophic relationships but incorporating more organic matter sources can delimit the uncertainty of mathematical mixing models by incorporating too many assumptions (Moseman *et al*. 2004). Underestimation of sources can lead to misinterpretation of entire structure of food web (Benstead *et al*. 2006). These results support the contention that aquaculture-derived organic matter fully subsidizes the diet of farmed sablefish and, to a lesser degree, the diets of other intended extractives.

Results from analysis of isotopic data have confirmed relative amounts of interspecies resource partitioning among organism groups sampled within an IMTA project site. There were no statistically significant temporal differences detected, possibly to do with consistent levels measured in fish feed signatures and with variable levels of reliance on fish feed as a diet source for samples. Furthermore, circular statistical metrics coupled with traditionally used dual isotope plots and multi-source mixing models provides a more dimensional and comprehensive investigation into the linkages that exist within marine food web dominated by an adjacent integrated multi-trophic aquaculture project site. The results of this investigation are recommended for use in the development of mathematical models to determine IMTA carrying capacities of intended extractive species. Development of enhanced models should lead to better control of economic and ecological sustainability in aquaculture.

Literature Cited

Aya, FA & Kudo, I (2007). Isotopic determination of Japanese scallop *Patinopecten (Mizuhopecten) yessoensis (jay)* tissues shows habitat-related differences in food sources. *J Shellfish Res* 26: 295-302.

Barrington K, Chopin T, Robinson S (2009). Integrated multi-trophic aquaculture (IMTA) in marine temperate waters. In D. Soto (ed.). Integrated mariculture: a global review. *FAO Fisheries and Aquaculture Technical Paper*. No. 529. Rome, FAO. pp. 7—46.

Batschelet E (1981). *Circular statistics in ecology*. Academic Press, New York.

Benstead JP, March JG, Fry B, Ewel KC, Pringle CM (2006). Testing IsoSource: stable isotope analysis of a tropical fishery with diverse organic matter sources. *Ecology* 87: 326—333.

Berger, MS & Jelinski, DE (2008). Spatial patterns of maternal investment in *Strongylocentrotus franciscanus* along a marine-terrestrial gradient. *Mar Ecol Prog Ser* 364: 119-127.

Bosley, KL & Wainright, SC (1999). Effects of preservatives and acidification on the stable isotope ratios (^{15}N:^{14}N, ^{13}C:^{12}C) of two species of marine animals. *Can J Fish Aquat Sci*: 56: 119-127.

Bustamante RH, Branch GM (1996). The dependence of intertidal consumers of kelp-derived organic matter on the west coast of South Africa. *J Exp Mar Biol Ecol* 196: 1—28.

Chopin T; Buschmann AH, Halling C, Troell M, Kautsky N, Neori A, Kraemer GP, Zertuche-González JA, Yarish C, Neefus C (2001). Integrating seaweeds into marine aquaculture systems: a key toward sustainability. *J Phycol* 37: 975—986.

Chopin T (2006). Integrated multi-trophic aquaculture. What it is and why you should care…and don't confuse it with polyculture. *Northern Aquaculture* 12: 4.

Darnaude AM, Salen-Picard C, Polunin NVC, Harmelin-Vivien ML (2003). Trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). *Ocealogia* 138: 325—332.

Dauby P, Mosora F, Vertez M (1990). A yearly study of $^{13}C/^{12}C$ isotopic ratio variations in the Calvi's Bay plankton. *Rapp Comm Int Explor Sci Mer Mediterr* 32: 201—202.

Deegan LA, Garritt RH (1997). Evidence for spatial variability in estuarine food webs. *Mar Ecol Prog Ser* 147: 31—47.

Dekar MP, Magoulick DD, Huxel GR (2009). Shifts in the trophic base of intermittent stream food webs. *Hydrobiologia* 635: 263—277.

De Niro MJ, Epstein S (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 42: 495—506.

Duarte P, Meneses R, Hawkins AJS, Zhu M, Fang J, Grant J (2003). Mathematical modelling to assess the carrying capacity for multi-species culture within coastal waters. *Ecolog Model* 168: 109—143.

Dubois S, Orvain F, Marin-Léal JC, Ropert M, Lefebvre S (2007). Small-scale spatial variability of food partitioning between cultivated oysters and associated suspension-feeding species, as revealed by stable isotopes. *Mar Ecol Prog Ser* 336: 151-160.

Ferreira JG, Hawkins AJS, Bricker SB (2006). Management of productivity, environmental effects and profitability of shellfish aquaculture—the Farm Aquaculture Resource Management (FARM) model. *Aquaculture* 264: 160—174.

Fry B, Sherr EB (1984). $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contrib Mar Sci* 27: 13—47.

Gannes LZ, O'Brien DM, del Rio CM (1997). Stable isotopes in animal ecology; assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78: 1271—1276.

Gao QF, Shin PKS, Guang-Hui L, Shi-Ping C, Cheung SG (2006). Stable isotope and fatty acid evidence for uptake of organic waste by green-lipped mussels *Perna viridis* in a polyculture fish farm system. *Mar Ecol Prog Ser* 317: 273—283.

Goering J, Alexander V, Haubenstock N (1990). Seasonal variability of stable carbon and nitrogen isotope ratios of organisms in a North Pacific Bay. *Estuar Coast Shelf Sci* 30: 239—260.

Grice AM, Loneragan NR, Dennison WC (1996). Light intensity and the interactions between physiology, morphology and stable isotope ratios in five species of sea grass. *J Exp Mar Biol Ecol* 195: 91—110.

Hemminga MA, Mateo MA (1996). Stable carbon isotopes in seagrasses: variability in ratios and use in ecological studies. *Mar Ecol Prog Ser* 140: 285—298.

Hobson KA, Clark RG (1992). Assessing avian diets using stable isotopes. I. Turnover of ^{13}C in tissues. *Condor* 94: 181—188.

Hobson KA, Piatt JF, Pitocchelli J (1994). Using stable isotopes to determine seabird trophic relationships. *J Anim Ecol* 63: 786—798.

Hsieh HL, Chen CP, Chen YG, Yang HH (2002). Diversity of benthic organic matter flows through polychaetes and crabs in a mangrove estuary: δ13C and δ34S signals. *Mar Ecol Prog Ser* 227: 145—155.

Iken, K, Brey, T, Wand, U, Voigt, J & Junghans, P (2001). Food web structure of the benthic community at the Porcupine Abyssal Plain (NE Atlantic): a stable isotope analysis. *Prog Oceanography*, 50, 383-405.

Jelinski DE, Krueger CC, Duffus, DA (2002). Geostatistical analyses of interactions between killer whales (Orcinus orca) and recreational whale-watching boats. *Appl Geog* 22: 393—411.

Kaehler S, Pakhomov EA (2001). Effects of storage and preservation on the $\delta^{13}C$ and $\delta^{15}N$ signatures of selected marine organisms. *Mar Ecol Prog Ser* 219: 299—304.

Kaehler S, Pakhomov EA, McQuaid CD (2000). Trophic structure of marine food web at the Prince Edward Islands (Southern Ocean) determined by $\delta^{13}C$ and $\delta^{15}N$ analysis. *Mar Ecol Prog Ser* 208: 13—20.

Kang CK, Sauriau PG, Richard P, Blanchard GF (1999). Food sources of the infaunal suspension-feeding bivalve *Cerastoderma edule* in a muddy sandflat of Marennes-Oléron Bay, as determined by analyses of carbon and nitrogen stable isotopes. *Mar Ecol Prog Ser* 187: 147—158.

Kharlamenko VI, Kiyashko SI, Imbs AB, Vyshkvartzev DI (2001). Identification of food sources of invertebrates from the seagrass Zostera marina community using carbon and sulfur stable isotope ratio and fatty acid analyses. *Mar Ecol Prog Ser* 220: 103—117.

Lajtha K, Michener R (2007). *Stable Isotopes in Ecology and Environmental Science*, 2nd ed. Wiley-Blackwell Publishing, pp. 592.

Layman CA, Arrington DA, Montana CG, Post DM (2007). Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology* 88: 42—48.

Lorrain A, Paulet Y-M, Chauvaud L, Savoye N, Donval A, Saout C (2002). Differential $\delta^{13}C$ and $\delta^{15}N$ signatures among scallop tissues: implications for ecology and physiology. *J Exp Mar Biol Ecol* 275: 47-61.

McKindsey CW, Thetmeyer H, Landry T, Silvert W (2006). Review of recent carrying capacity models for bivalve culture and recommendations for research and management. *Aquaculture* 261: 451—462.

Michener RH, Schell DM (1994). *Stable isotope ratios as tracers in marine aquatic food webs*. In: Lajtha K, Michener RH (eds) Stable isotopes in ecology and environmental sciences. Blackwell, Oxford, pp 138—157.

Minagawa M, Wada E (1984). Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between ^{15}N and animal age. *Geochim Cosmochim Acta* 48: 1135—1140.

Minagawa M, Wada E (1986). Nitrogen isotope ratios of red tide organisms in the East China Sea: A characterization of biological nitrogen fixation. *Mar Chem* 19: 245—259.

Moseman SM, Levin LA, Currin C, Forder C (2004). Colonization, succession, and nutrition of macrobenthic assemblages in a restored wetland at Tijuana Estuary, California. *Estuar Coast Shelf Sci* 60: 755—770.

Nadon M-O, Himmelman JH (2006). Stable isotopes in subtidal food webs: Have enriched carbon ratios in benthic consumers been misinterpreted? *Limnol Oceanogr* 51: 2828—2836.

Perga, ME & Gerdeaux, D (2005). 'Are fish what they eat' all year round? *Oecologia*, 144: 598-606.

Peterson BJ, Fry B (1987). Stable isotopes in ecosystem studies. *Annu Rev Ecol Syst* 18: 293—320.

Phillips DL (2001). Mixing models in analyses of diet using multiple stable isotopes: a critique. *Oecologia* 127: 166—170.

Phillips DL & Gregg JW (2003). Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136: 261—269.

Pinnegar JK & Polunin NVC (1999). Differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. *Funct Ecol*: 225-231.

Polis GA, Power ME, Huxel GR Eds. (2004). *Food webs at the landscape level*. Univ of Chicago Press, Chicago.

Quan W, Fu C, Jin B, Luo Y, Li B, Chen J, Wu J (2007). Tidal marshes as energy sources for commercially important nekton organisms: stable isotope analysis. *Mar Ecol Prog Ser* 352: 89—99.

Rau G (1978). Carbon-13 depletion in a subalpine lake: carbon flow implications. *Science* 201: 901—902.

Reid GK, Liutkus M, Robinson SMC, Chopin TR, Blair T, Lander T, Mullen J, Page F, Moccia RD (2009). A review of the biophysical properties of salmonid faeces: implications for aquaculture waste dispersal models and integrated multi-trophic aquaculture. *Aquacul Res* 40: 257—273.

Riera P, Richard P (1997). Temporal variation of $\delta^{13}C$ in particulate organic matter and oyster *Crassostrea gigas* in Marennes-Oléron Bay (France): effect of freshwater. *Mar Ecol Prog Ser 147*: 105—115.

Riera P, Stal LJ, Nieuwenhuize J (2002). $\delta^{13}C$ vs $\delta^{15}N$ of co-occurring molluscs within a community dominated by *Crassostrea gigas* and *Crepidula fornicata* (Oosterschelde, The Netherlands). *Mar Ecol Prog Ser* 240: 291—295.

Sarà G, Scilipoti D, Milazzo M, Modica A (2004). Effects of fish farming waste to sedimentary and particulate organic matter in a southern Mediterranean area (Gulf of Castellammare, Sicily): a multiple stable isotope study ($\delta^{13}C$ and $\delta^{15}N$). *Aquaculture* 234(1-4): 199—213.

Sarakinos HC, Johnson ML, Vander Zanden MJ (2002). A synthesis of tissue preservation effects on carbon and nitrogen stable isotope signatures. *Can J Zool* 80: 381—387.

Schindler DE, Lubetkin SC (2004). Using stable isotopes to quantify material transport in food webs. Pages 25—42 *in* Polis GA, Power ME, Huxel GR (*eds*) *Food webs at the landscape level*. University of Chicago Press, Chicago, Illinois, USA.

Schmidt SN, Olden JD, Solomon CT, Vander Zanden MJ (2007). Quantitative approaches to the analysis of stable isotope food web data. *Ecology* 88: 2793—2802.

Schmidt SN, Vander Zanden MJ, Kitchell JF (2009). Long-term food web change in Lake Superior. *Can J Fish Aquat Sci* 66: 2118—2129.

Simenstad, CA & Wissmar, RC (1985). $\delta^{13}C$ evidence of the origins and fates of organic carbon in estuarine and nearshore food webs. *Mar Ecol Prog Ser* 22: 141-152.

Smith BN, Epstein S (1971). Two categories of $^{13}C/^{12}C$ ratios for higher plants. *Plant Physiol* 47: 380—384.

Sutherland TF, Martin AJ, Levings CD (2001). Characterization of suspended particulate matter surrounding a salmonid net-pen in the Broughton Archipelago, British Columbia. *ICES* 58: 404—410.

Sweeting CJ, Jennings S, Polunin NVC (2005). Variance in isotopic signatures as a descriptor of tissue turnover and degree of omnivory. *Funct Ecol* 19: 777—784.

Troell M, Joyce A, Chopin T, Neori A, Buschmann AH, Fang J-G (2009). Ecological engineering in aquaculture—Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. *Aquaculture* 297: 1—9.

Vander Zanden MJ, Chandra S, Allen BC, Reuter JE, Goldman CR (2003). Historical food web structure and restoration of native aquatic communities in the Lake Tahoe (California—Nevada) Basin. *Ecosystems* 6: 274—288.

Vander Zanden MJ, Olden JD, Gratton C (2006). Food-web approaches in restoration ecology, Pages 165—189 *in* Falk DA, Palmer MA, Zedler JB eds. *Foundations in restoration ecology*. Island Press, Washington DC, USA.

Vizzini S, Mazzola A (2002). Stable carbon and nitrogen ratios in the sand smelt from a Mediterranean coast area: feeding habits and effect of season and size on feeding strategy. *J Fish Biol* 60: 1498—1510.

Vizzini S, Mazzola A (2003). Seasonal variations in the stable carbon and nitrogen isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of primary producers and consumers in a western Mediterranean coastal lagoon. *Mar Biol* 142: 1009—1018.

Vizzini S, Mazzola A (2006). Sources and transfer of organic matter in food webs of a Mediterranean coastal environment: evidence for spatial variability. *Estuar Coast Shelf Sci* 66: 459—467.

Wada E, Kadonaga T, Matsuo S (1975). ^{15}N abundance in nitrogen of naturally occurring substances and global assessment of denitrification from isotopic viewpoint. *Geochem J* 9: 139—148.

Zar JH (1999). *Biostatistical analysis*, 4th ed. Prentice-Hall, Upper Saddle River, NJ.

4.0 GENERAL CONCLUSIONS

4.1 Discussion and 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 (see review by Michener & Schell 1994). Marine communities are ideal research models in that physical and biological interactions occur over numerous spatial-temporal scales (Link 2002). They are subject to a wide array of environmental conditions and disturbances. Research contributions to the scientific community demonstrate efforts to ensure appropriate monitoring and protection of marine ecosystems and biodiversity.

Analyzing a marine benthic community situated within and adjacent to 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 attempts to balance four extractive species, representing both marine producers and consumers: sablefish *Anoplopoma fimbria*, blue mussels *Mytilus edulis*, Pacific scallops *Patinopectin caurinus*, and sugar kelp *Saccharina latissima*. In all IMTA facilities, project leaders choose extractives based on documented husbandry practices, habitat suitability (*ie*. appropriate role within adjacent ecosystem), biomitigation capacity, and economic value (Barrington *et al*. 2009). IMTA sites are spatially manageable and abundantly reared populations of extractives permit replicability. Furthermore, residency of aquaculture facilities in one location permits longer term temporal examination of community dynamics.

This thesis represents my investigation in to the influence of IMTA organic effluent waste on intended extractive and unintended biofouling species within and adjacent to the facility. I used stable carbon and nitrogen isotope analysis to establish trophic linkages between extractive and biofouling species adjacent to the IMTA

facility. I acquired data for a temporal time period of approximately one year in order to assess possible monthly fluctuations in isotopic signatures. Additionally, I used an isotopic data subset that compared signatures of two species (blue mussels *Mytilus edulis* and brooding transparent tunicates *Corella inflata*) found both adjacent to the IMTA site and at a reference site located 500 m away. This was an effort to measure the presence of any effect on the diets of IMTA derived organic waste on biofouling species including the potential for intra- and/or interspecific competition. I made these predictions:

1) *Mytilus edulis* and *Corella inflata* species sampled within the IMTA site would have enriched carbon and nitrogen isotopic signatures that, if assimilated, would reflect this organic waste subsidy. Furthermore, these signatures should be more enriched than those found within the tissues of the same species sampled at the reference site.
2) Resource partitioning models would detect IMTA-generated waste to be the primary source of nutrient assimilated by samples collected adjacent to facility, as compared to average POM data gathered in the literature.
3) Temporal fluctuations in signatures among IMTA site sampled species would be minimal as aquaculture derived organic effluent is considered a constant potential subsidy.

Enriched isotopic signature results for blue mussels *M. edulis* sampled at the IMTA facility agreed with my first prediction; however a similar outcome was not met for brooding transparent tunicates *C. inflata*. Furthermore, the signature levels detected in *C. inflata* were not significantly different from those measured at the reference site. Despite growing in relatively high abundances adjacent to the sablefish cages, there was no evidence to conclude that the diets of *C. inflata* are directly influenced by IMTA-derived organic waste. This result was corroborated by Dubois *et al*. (2007) who measured the signatures of several co-occurring suspension feeding epibionts, adjacent to cultivated oysters, and found the ascidian *Ascidiella aspersa* to consistently exhibit limited intraspecific variability in signatures regardless of sampling site or composition of available food source. They propose

that *A. aspersa* are less capable of trophic plasticity than other suspension feeders, either assimilating proportionately less IMTA-derived waste as food, or another food source altogether (eg. particulate organic matter (POM)).

Among the IMTA-site species examined, IMTA-derived effluent (fish feed and fish faeces) was the least represented proportionately within the diets of brooding transparent tunicates *C. inflata*. This is followed by the other two tunicate species sampled, hairy (*Boltenia villosa*) and broadbase (*Cnemidocarpa finmarkeinsis*) tunicates that, although from the same feeding guild, are also not likely to be competing with intended extractives due to relatively low organic waste proportionately represented. Blue mussels *M. edulis* exhibited lower than expected proportionate values which are similar to the results obtained by Gao *et al*. (2006), who analyzed food partitioning of green-lipped mussels *Perna viridis* adjacent to fish farms. They propose that proportions of aquaculture-derived organic matter could be higher in systems that use powdered fish meal (see experiment by Yokoyama *et al*. 2002) thereby enhancing accessibility and digestibility to mussels via direct deposition. In accordance with the second prediction, the highest degree of proportion of aquaculture-derived waste were assimilated into the diets of sablefish *A. fimbria*, sea urchins *S. franciscanus*, and Pacific scallop *P. yessoensis*.

Particular directional changes in isotopic values for IMTA-site species examined were not detected through circular statistical analysis, which supports the third prediction. Other related studies have employed circular matrices to examine isotopic responses to large scale disturbances, such as introduction of non-native species to focal ecological communities, and detect specific directional changes of total species averages in longer time scales. To date, my study was the first to employ this method within an IMTA facility and in the course of one year.

4.2 Limitations of Analysis

The isotopic signatures of additional potential food sources were intended to be sampled for this study. These include marine and terrestrial particulate organic matter (in lieu of previously published data), benthic macro- and microalgae, and

detritus material at the seafloor (below IMTA facility). Including the isotopic results of these materials would likely have led to more conclusive results as to the resource partitioning modeling analysis for each species. Out of the three food sources identified in this study, only two of them were measured directly at this site. *In situ* sampling of marine particulate organic matter would have been enormously valuable in to the relative contribution of the diets of consumers sampled in this study. Much caution is emphasized when approaching interpretations of the marine POM averages used in Chapters 2 and 3. Given the relatively high amount of variation of nitrogen isotopic signature averages, these data can only serve as possible non-IMTA food source, rather than represent realistic values for marine POM signatures within Kyuquot Sound, BC. With temperate waters seasonally variable in temperature and thus CO_2, it would be advantageous to measure and present consequent seasonal isotopic fluctuations of marine POM that would likely, in turn, affect the isotopic values of higher trophic consumers that feed on it (Wainright & Fry 1994). Peterson & Fry (1987) provide an estimation of global isotopic averages of carbon and nitrogen signatures of marine POM. They report $\delta^{13}C$ range of about -19 to -24 ‰ and a much wider $\delta^{15}N$ range of about -2 to 11 ‰. Despite falling within these ranges, had the calculated isotopic averages from the literature been different would have resulted in different hypothetical outcomes for the food partitioning exercises in both Chapters 2 and 3.

The following Figures (4.1 and 4.2) are food partitioning results from IsoSource using the same isotopic signatures for consumers blue mussels and brooding transparent tunicates obtained from the present study but with different hypothetical carbon and nitrogen values for marine POM. Figure 4.1 depicts how results change when the most depleted value for $\delta^{13}C$ (-24 ‰) and the most enriched value for $\delta^{15}N$ (11 ‰), as reported by Peterson & Fry (1987), are inputted. This could be a possible scenario for winter. Figure 4.2 features the most enriched value for $\delta^{13}C$ (-19 ‰) and the most depleted value for $\delta^{15}N$ (-2 ‰), also reported by Peterson & Fry (1987). This could be a possible scenario for summer.

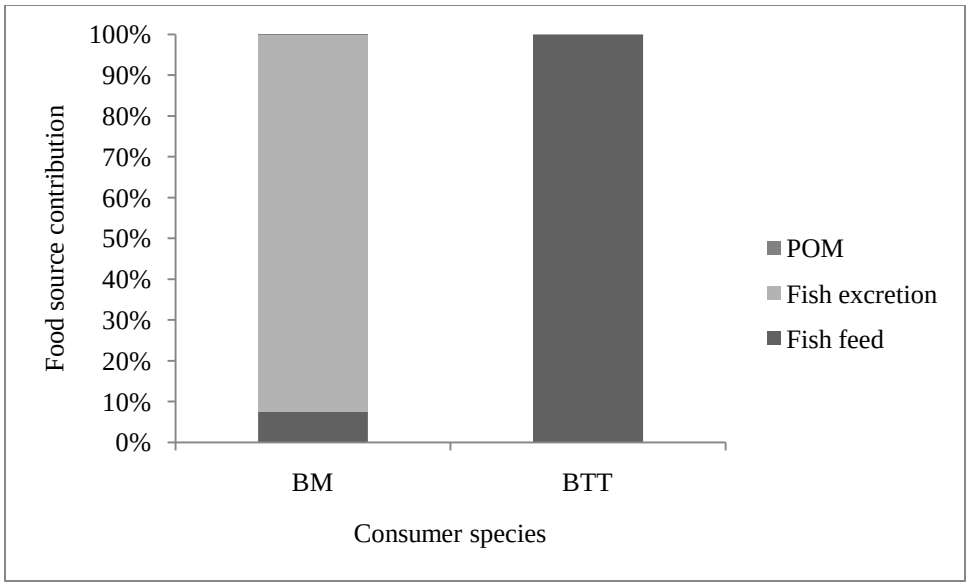

Figure 4.1 Histogram of interspecific variability of average relative contributions (%) of food sources for each sampled consumer species as estimated by 3-source mixing model software IsoSource 1.3.1. Food sources include hypothetic values for marine POM (most enriched for $\delta^{13}C$ and most depleted for $\delta^{15}N$, as reported by Peterson & Fry (1987)), fish excretion, and fish feed. Abbreviations of consumer species are BM, blue mussel (*Mytilus edulis*), and BTT, brooding transparent tunicate (*Corella inflata*).

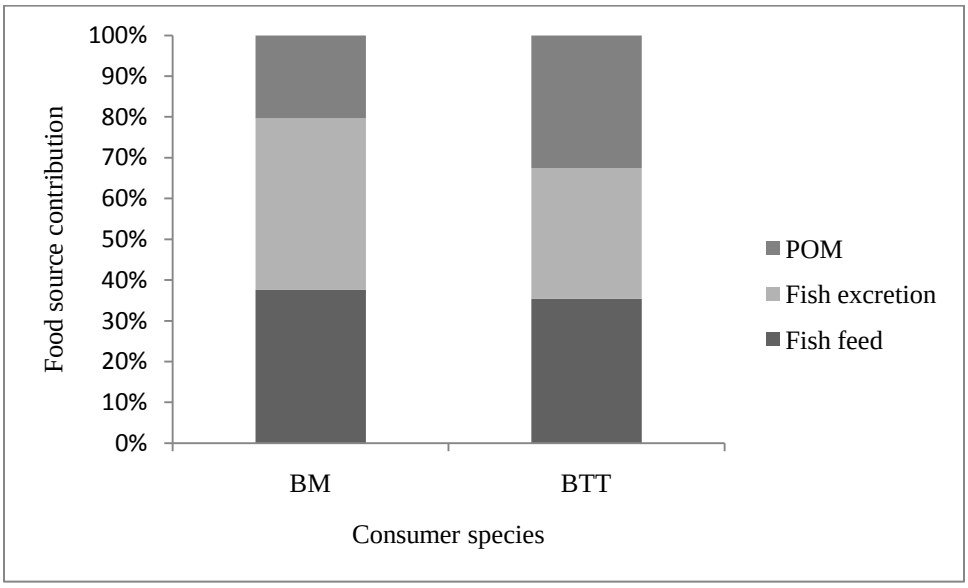

Figure 4.2 Histogram of interspecific variability of average relative contributions (%) of food sources for each sampled consumer species as estimated by 3-source mixing model

software IsoSource 1.3.1. Food sources include hypothetic values for marine POM (most depleted for $\delta^{13}C$ and most enriched for $\delta^{15}N$, as reported by Peterson & Fry (1987)), fish excretion, and fish feed. Abbreviations of consumer species are BM, blue mussel (*Mytilus edulis*), and BTT, brooding transparent tunicate (*Corella inflata*).

Figures 4.1 and 4.2 demonstrate how different outcomes may result in consumer food resource partitioning had different carbon and nitrogen isotopic signatures within the range of marine POM were inputted within the partitioning model. In all cases, including what was reported in Chapters 2 and 3, IMTA-derived material contributes the most to the diets of mussel *M. edulis*. A different outcome, however, has resulted for tunicate *C. inflata*, where IMTA-derived material contributes largely to tunicate diets given these hypothetical signatures for marine POM, the opposite of what was reported in Chapters 2 and 3. Though the hypothetic values for marine POM were at extreme ends of the reported range, these scenarios are not unlikely when making seasonal measurements. A more thorough understanding of baseline isotopic signatures of food resources can greatly assist in understanding trophic relationships but incorporating more organic matter sources can delimit the uncertainty of mathematical mixing models by incorporating too many assumptions (Moseman *et al*. 2004). Underestimation of sources can lead to misinterpretation of entire structure of food web (Benstead *et al*. 2006). Certainly, wastes released from the IMTA facility could be intercepted by species that were not considered in this study. Abiotic processes occurring at both the IMTA facility and the reference site could have also been considered as a compliment to the seasonal isotopic data gathered. These could include chlorophyll *a*, water temperature and salinity, and current speed.

Fish feed was only sampled once. I had possibly falsely assumed that isotopic signatures of fish feed were fixed and subsequently neglected to test this assumption. In retrospect I should have taken fish feed samples each time month—however the temporal variation of isotopic signatures of fish faeces was slight. Sablefish *A. fimbria* were fed with Taplow commercial aquafeed formula containing 62 %

protein sourced from fish meal and 100 % oil derived from fish oil (Taplow 2011). Generally speaking, commercial aquafeeds are produced from fishmeal and other fishery derivatives (eg. fish oil, fish protein hydrolysates) that provide protein, energy, essential fatty acids and minerals (Tacon 1994). Most often, marine by-products include whole bodies and/or parts of small fishes are incorporated into aquafeeds; however some feeds are comprised of terrestrial protein derivatives such as poultry by-products, cottonseed and peanut (Tacon & Metian 2008). Reformulation of fishery by-products and oil constituents with terrestrial sources into the production of aquafeed has become increasingly more common. Friesen (2008) analyzed the effects of alternative dietary lipids and proteins on Atlantic salmon (*Salmo salar*) and sablefish (*A. fimbria*) and found minimal negative effects on growth and quality, thereby lending positive support to the industry trend of aquafeed reformulation. In doing so, isotopic signatures of fish feed may not be statistically significantly different from natural food source signatures for suspension feeders, thereby reducing the utility of stable isotope analysis for detecting of aquaculture-derived organic waste.

Sampling co-cultured and biofouling species from a gradient of locations and depths may provide more confidence that sampling occurred directly down current from the sablefish cages. Furthermore, it is not certain that the placement of the reference site, 500 m from the IMTA facility, was far enough to avoid any influence from any aquacultural activity. Other studies have measured effects from fish farms several hundred meters away. An ideal site would share similar hydrodynamic processes and physical characteristics, which can be challenging. Gathering more samples recruited from a reference site, and over a longer temporal period, would have many advantages in terms of more conclusive multiple comparisons with like organisms from the IMTA facility. Furthermore, this data could serve to provide more confidence in the results of multi-source mixing models of resource portioning in consumer diets if models could also represent naturally occurring samples. The presence or absence of inter- or intra-specific competition could also result.

4.3 Future Contributions

Given the dearth of research that is associated with IMTA facilities to date, there are numerous directions with which to take future research. Previous studies involving the influence of aquaculture-related effluent on co-cultured extractives and surrounding marine benthic and intertidal communities have considered additional effects. These include increased growth and production of filter feeders (Wallace 1980; Jones & Iwama 1991), increased phytoplankton and POM biomass (Jones & Iwama 1991; Mazzola & Sarà 2001), carrying capacity modeling (Carver & Mallet 1990; Duarte *et al*. 2003; Grant *et al*. 2007), sulfur isotopes and fatty acid profiling (Gao *et al*. 2006; Frederiksen *et al*. 2007). Measuring these effects in addition to stable isotopes analysis of co-cultured extractives and adjacent biofouling and benthic community species would provide a better understanding of the impacts of IMTA. Furthermore, I would recommend that this study be replicated and expanded over a longer time span, in order to make clearer inferences as to the seasonal processes occurring in Kyuquot Sound. Large scale spatial variation could also be factored by conducting a comparative analysis with other temperate IMTA sites, including a facility headed by Theirry Chopin and Shawn Robinson located in Bay of Fundy, Canada (Chopin *et al*. 2004).

There is evidence from this study to support the sustainability of IMTA within Kyuquot Sound, BC. Stable carbon and nitrogen isotope analysis were informative tracers of IMTA-derived organic effluent assimilated in tissues of adjacent intended extractive and biofouling community species. Multi-source mixing models provided insights as to the level of resource partitioning occurring, and permitted inferences to be made regarding any intra- and/or interspecific competition taking place among species within this marine community. To date this study was the first to use circular matrices to measure any directional change in isotopic composition on a seasonal scale within an IMTA facility, which allowed quantitative interpretation of qualitative dual isotope plots.

To summarize, food source partitioning among intended extractive species and biofouling communities adjacent to IMTA is variable. Fish feed and faeces were found to be primary sources of food assimilated into the tissues of most intended extractive species whereas these sources were proportionately less represented in the tissues of biofouling epibionts sampled. Therefore, competition for resources is not likely to be occurring. Implications of this research include the importance of IMTA-derived waste as a food source to support the sustainability of IMTA. Evidence of relative uptake and assimilation indicate varying degrees of reliance of IMTA to subsidize adjacent marine communities. Furthermore, stable isotopes analysis did not produce evidence of statistically significant temporal variation in sample signatures, which could also indicate a relatively stable system with which to model for carrying capacity. Overall this research shall be included with others that strive towards greater understanding of the many aspects of aquaculture-derived waste as subsidies to surrounding marine communities, as well as support the exploration into sustainable means to resolving global fisheries demands.

Literature Cited

Barrington K, Chopin T, Robinson S (2009). Integrated multi-trophic aquaculture (IMTA) in marine temperate waters. In: Soto D (ed) Integrated mariculture: a global review. *FAO Fisheries and Aquaculture Technical Paper*. No. 529 Rome, FAO. Pp 7—46.

Benstead JP, March JG, Fry B, Ewel KC, Pringle CM (2006). Testing IsoSource: stable isotope analysis of a tropical fishery with diverse organic matter sources. *Ecology* 87: 326—333.

Carver CEA, Mallet AL (1990). Estimating the carrying capacity of a coastal inlet for mussel culture. *Aquaculture* 88: 39—53.

Chopin T, Robinson S, Sawhney M, Bastarache S, Belyea E, Shea R, Armstrong W, Stewart I, Fitzgerald P (2004). The AquaNet integrated multi-trophic aquaculture project rationale of the project and development of kelp cultivation as the inorganic extractive component of the system. *Bull Aqua Assn Can* 104: 11—18.

Duarte P, Meneses R, Hawkins AJS, Zhu M, Fang J, Grant J (2003). Mathematical modelling to assess the carrying capacity for multi-species culture within coastal waters. *Ecol Model* 168: 109—143.

Dubois S, Orvain F, Marin-Léal JC, Ropert M, Lefebvre S (2007). Small-scale spatial variability of food partitioning between cultivated oysters and associated suspension-feeding species, as revealed by stable isotopes. *Mar Ecol Prog Ser* 336: 151—160.

Frederiksen MS, Holmer M, Díaz-Almela E, Marba N, Duarte CM (2007). Sulfide invasion in the seagrass *Posidonia oceanica* at Mediterranean fish farms: assessment using stable sulfur isotopes. *Mar Ecol Prog Ser* 345: 93—104.

Freisen E (2008). Use of alternative feed ingredients and the effects on growth and flesh quality of Atlantic salmon (*Salmo salar*) and sablefish (*Anoplopoma fimbria*). University of British Columbia. PhD.

Gao Q-F, Shin PKS, Lin G-H, Chen S-P, Cheung SG (2006). Stable isotope and fatty acid evidence for uptake of organic waste by green-lipped mussels *Perna viridis* in a polyculture fish farm system. *Mar Ecol Prog Ser* 317: 273—283.

Grant J, Curran KJ, Guyondet TL, Tita G, Bacher C, Koutitonsky V, Dowd M (2007). A box model of carrying capacity for suspended mussel aquaculture in Lagune de la Grande-Entrée, Iles-de-la-Madeleine, Québec. *Ecol Model* 200: 193—206.

Jones T, Iwama G (1991). Polyculture of the Pacific oyster, *Crassostrea gigas* (Thunberg), with Chinook salmon *Oncorhynchus tshawytscha*. *Aquaculture* 92: 313—322.

Link J (2002). Does food web theory work for marine ecosystems? *Mar Ecol Prog Ser* 230: 1—9.

Mazzola A, Sarà G (2001). The effect of fish farming organic waste on food availability for bivalve molluscs (Gaeta Gulf, Central Tyrrhenian, MED): stable carbon isotopic analysis. *Aquaculture* 192: 361—379.

Michener RH, Schell DM (1994). Stable isotope ratios as tracers in marine aquatic food webs. In: Lajtha K, Michener RH (eds) *Stable isotopes in ecology and environmental science*. Blackwell Science, London, p 138—157.

Moseman SM, Levin LA, Currin C, Forder C (2004). Colonization, succession, and nutrition of macrobenthic assemblages in a restored wetland at Tijuana Estuary, California. *Estuar Coast Shelf Sci* 60: 755—770.

Peterson BJ, Fry B (1987). Stable isotopes in ecosystems studies. Ann Rev Ecol System 18: 293—320.

Tacon AGJ (1994). Feed ingredients for carnivorous fish species: alternatives to fishmeal and other fishery resources. *FAO Fisheries Circular no. 881*, Food and Agricultural Organisation of the United Nations, Rome, Italy.

Tacon AGJ, Metian M (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285: 146—158.

Taplow (2011). Retrieved February 23, 2011, from http://www.taplow.com.

Wainright SC, Fry B (1994) Seasonal variation of the stable isotopic compositions of coastal marine plankton from Woods Hole, Massachusetts and Georges Bank. Estuaries 17: 552—560.

Wallace E, Hattori A (1978). Natural abundance of ^{15}N in particulate matter in the North Pacific Ocean. *Geochim Cosmochim Acta* 40: 249—251.

Yokoyama H, Higano J, Adachi K, Ishihi Y, Yamada Y, Pichitkul P (2002). Evaluation of shrimp polyculture system in Thailand based on stable carbon and nitrogen isotope ratios. *Fish Sci* 68: 745—750.