SPATIAL AND TEMPORAL VARIATION IN KELP-DERIVED DETRITUS AND ITS DIETARY IMPORTANCE TO CONSUMERS ALONG THE WEST COAST OF VANCOUVER ISLAND, CANADA

by

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Abstract

Stable isotope analysis was used to determine spatial and temporal patterns of suspended kelp-derived detritus (KDD) and its contribution to consumers along a gradient of kelp abundance driven by recovering sea otter (*Enhydra lutris*) populations along the west coast of Vancouver Island (WCVI). During the summer and winter, ocean surface size-fractionated particulate organic matter (POM), dominant kelp species (order Laminariales), surface plankton and benthic organisms were sampled along offshore transects (0 – 30 km), and analyzed for carbon and nitrogen stable isotopes. Phytoplankton isotope fractionation characteristics were utilized along with principal component analysis to determine seasonally and size fraction specific $\delta^{13}C$ and $\delta^{15}N$ values. Blooming size fractions of phytoplankton were enriched in ^{13}C by 2.5 to 5.4 ‰ and enriched by 0.3 to 3.1 ‰ in ^{15}N. There were significant within and among region, and between season differences in kelp isotopic values. For example, during the summer, the otter-present *Macrocystis pyrifera* mean $\delta^{13}C$ value (-13.17 ± 1.12 ‰) was more enriched than *Nereocystis luetkeana* (-16.89 ± 1.88 ‰; $p < 0.001$). These results were used in a Bayesian isotope mixing model (MixSIR) to estimate KDD contributions. The contribution to POM showed the greatest variability with distance from the kelp forest within the otter-absent region. However, in general, there was little difference among regions with respect to KDD contribution. Seasonally, KDD contribution to POM was greater during the summer. KDD contribution to total POM and 20 – 63 µm POM was similar and generally greater than 0.7 – 20 µm POM.

Modeled estimates of KDD contribution to benthic invertebrates and fish were high (> 40 %) and similar with size, among regions and between seasons, with the exception of red turban snails (*Astraea gibberosa*) in the otter-present region where KDD contribution was 41.2 to 96.6 % lower than the otter-absent region.

These results indicate that kelp abundance is not the only driver of KDD dispersal spatially and temporally. Factors such as local oceanography, kelp forest community composition, large variation in kelp isotope values, and similarity between kelp and phytoplankton isotope values may affect these patterns and lead to high uncertainty in modeled KDD contributions.

Preface

The research presented in this thesis is to complete part of Objective 3 of the British Columbia Coastal Ecosystem Services amongst Trophic Cascades Strategic Project. Evgeny A. Pakhomov (Earth and Ocean Sciences, University of British Columbia) designed this study in collaboration with Kai M.A. Chan (Institute of Resources, Environment and Sustainability, University of British Columbia), Chris D.G. Harley (Zoology, University of British Columbia) and Jonathan B. Shurin (Section of Ecology, Behavior and Evolution, University of California, San Diego).

Larysa Pakhomov, Chris Payne, Sven Kaehler and E.A.P. assisted with data collection. E.A.P. and Russell W. Markel (Zoology, University of British Columbia) edited the manuscript.

Data collection and analyses, and writing for this manuscript were performed by Brock C. Ramshaw.

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Dedication

“For this sacred land./It has seen many hands./It has wealth and gold./Yet it is fragile and old./And all the greedy souls./Just don't care to know./Of the changes it will confront./So speak out loud./Of the things you are proud./And if you love this coast./Then keep it clean as it hopes./'Cause the way that it shines./May just dwindle with time./With the changes it will confront.” – *Messages* – Xavier Rudd

“Everything is free now./That's what they say./Everything I ever done./Gonna give it away./Someone hit the big score./They figured it out./They were gonna do it anyway./Even if doesn't pay./I can get a tip jar./Gas up the car./Try to make a little change./Down at the bar./Or I can get a straight job./I've done it before./Never minded working hard./It's who I'm working for.” – *Everything is Free* – Gillian Welch

“Unlike so many things you've never let me down./Never steered me wrong./And with every passing day./Distill my dedication./With you I've never felt alone./And as I stumble through these days./I hope you'll understand./It's so much more than a phase./It's everything I am./When I'm straying in darker times./When I'm feeling left behind, you're always there./The music somehow seems to get me by.” – *Libertine* – Good Riddance

“Hey man of science with your perfect rules of measure./Can you improve this place with the data that you gather?/Hey Mother Mercy can your loins bear fruit forever?/Is your fecundity a trammel or a treasure?.../And I want to conquer the world./Give all the idiots a brand new religion./Put an end to poverty, uncleanliness and toil./Promote equality in all of my decisions./With a quick wink of the eye./And a "God you must be joking!"./I want to conquer the world./Expose the culprits and feed them to the children./I'll do away with air pollution and then I'll save the whales./We'll have peace on earth and global communion.” – *I Want to Conquer the World* – Bad Religion

Chapter 1: Introduction

Vancouver Island, British Columbia, Canada (49° 24'29"N,125° 25'19"W) is situated in the northeast Pacific Ocean at the northern extent of the California Current Large Marine Ecosystem (Sherman and Alexander 1986). This temperate ecosystem is highly productive with highly seasonal upwelling, phytoplankton production and currents (Thomson 1981, Thomson et al. 1989). Due to variation in the seasonal intensity of upwelling which provides nutrients for phytoplankton and macroalgae (Dugdale 1985), the base of complex food webs that include migrating juvenile and adult salmon, whales, seabirds, and sea otters. The following research was conducted on the west coast of Vancouver Island (WCVI), from Kyuquot to Barkley Sound, a stretch of coastline that has recently undergone a dramatic ecological perturbation, the recovery of the sea otter (*Enhydra lutris*), a well-known keystone predator (*sensu* Paine 1966, Estes and Palmisano 1974).

1.1 The extirpation, reintroduction and ecological role of sea otters

Sea otters (*Enhydra lutris*) had a historical range from northern Japan to the Baja of California, Mexico (Nichol et al. 2005). In the 18th and 19th centuries sea otters were hunted extensively and extirpated from many areas of their entire range as part of the maritime fur trade, which included the entire coast of British Columbia, Canada by 1929 but were commercially, and likely ecologically, extinct by the late 19th century (Cowan and Guiguet 1960, Kenyon 1969). Between 1969 and 1972 eighty-nine sea otters were reintroduced from Alaska to Checleset Bay on the WCVI in an attempt to re-establish populations within B.C. (Bigg and MacAskie 1978). Since this time population growth has been at a rate of 8 to 19 % per year (1995 to 2004, 1977 to 1995, respectively; Watson et al. 1997, Nichol et al. 2005). Along with this population growth, their range has expanded north-westwards and south-eastwards along the WCVI to include roughly 2,673 individuals (as of 2001; Nichol et al. 2005). The current population occupies 25 – 33 % of the historical distribution in British Columbia (Nichol et al. 2005) with the expectation of an increased range and re-establishment in optimum habitat (Gregr et al. 2008).

Sea otters occupy exposed, rocky reef areas along the WCVI where they can forage up to 40 m in depth, but can dive up to 100 m in depth (Estes et al. 2003, Bodkin et al. 2004, Nichol et al. 2005). In areas where they have recently established they prefer easily-accessible prey such as sea urchins before diversifying their diet to include bivalves, snails, chitons, mussels, crabs, sea stars and occasionally fish (Estes et al. 1982). Sea otters are a “keystone” predator (*sensu* Paine

1966), and as a result have a disproportionately large effect on their environment relative to their abundance and determine the types and numbers of other species in the nearshore community (Estes and Palmisano 1974). Estes and Palmisano (1974) describe the reduction of sea urchin populations at islands where sea otters have re-established and identify sea otter presence as the primary driver for differences in the nearshore community between islands with and without sea otters, specifically the overwhelming abundance of macroalgae in areas with sea otters and vice versa. This "top-down" trophic cascade (Paine 1980, Pace et al. 1999) frees macroalgae from herbivory and leads to the proliferation of dense kelp forests, and their associated invertebrate and fish communities (Breen et al. 1982, Watson 1993, Estes and Duggins 1995), which supply three dimensional habitat, and a direct and indirect food source through grazing and the ingestion of kelp-derived particulate organic matter (POM; Dayton 1985, Duggins et al. 1989). Primary production and productivity can also be limited by abiotic processes (Dayton 1985).

1.2 Coastal phytoplankton production

Coastal upwelling brings cold and nutrient rich deep water to the surface where it supplies primary producers with essential nutrients for photosynthesis (Dugdale 1985). Most of the ocean based photosynthesis occurs along the coastal margins, which occupy only 0.1% of the total ocean area, and has been estimated to support phytoplankton productivity in upwelling regions ranging from 200 to 973 g C m^{-2} yr^{-1} (Pauly and Christensen 1995, Hahm and Kim 2001). Phytoplankton concentrations along the WCVI reach a maximum during late spring to mid-summer when solar radiation is high and nutrients are readily available from winter mixing of the water column and upwelling (Parsons and Lalli 1988, Whitney et al. 1998). Planktonic biomass and productivity can be highly localized and ephemeral in frontal zones or in upwelling plumes (Steele 1978). Phytoplankton species assemblages over WCVI's continental shelf predominantly consist of diatoms (mainly *Chaetoceros debilis* and *Leptocylindrus danicus*, but *Pseudonitzschia* spp., *Skeletonema costatum*, *Asterionella glacialis*, and *Dactyliosolen fragillissimus* are also present) with a significant contribution of nanoflagellates (Tortell et al 2000, Harris 2001). Diatoms are thought to outcompete other species during periods of intense upwelling (Harris 2001); however, phytoplankton community assemblages are known to fluctuate on inter-annual and decadal scales (Barton et al 2003, Leterne et al 2005).

1.3 Kelp productivity

Benthic marine algae are comprised of three major groups: Phaeophyceae (brown), Chlorophyceae (green) and Rhodophyceae (red). In temperate coastal areas the greatest productivity and biomass is dominated by the brown algae in the orders Laminariales and Fucales (Dayton 1985). Laminariales are referred to as kelps, and are large fleshy macroalgae that occupy low intertidal and shallow subtidal rocky reef (Dayton 1985). In British Columbia, three relatively abundant species include the perennials, giant kelp *Macrocystis pyrifera*, and old

growth kelp *Pterygophora californica*, and one annual species, bull kelp *Nereocystis luetkeana* (Druehl 2002; Martone and Markel, in prep). Similar to phytoplankton, kelp productivity studies have highlighted the importance of light, nutrients (nitrogen in particular), and temperature (Mann 1982, Dayton 1985). During the summer, photosynthesis and carbon storage is at a maximum while nutrients are at a minimum (Mann 1982). Therefore, when dissolved nitrate increases during the fall due to increased vertical mixing and decreased nutrient utilization by phytoplankton, kelps grow rapidly and utilize their stored carbon (Mann 1982). When sea surface temperatures decrease in the winter photosynthate accumulates and is utilized during spring growth (Mann 1982). *Macrocystis* in California has been shown to store dissolved nitrogen for a few weeks and then use for growth (e.g. Gerard 1982).

Kelp-derived productivity contributes substantially to nearshore primary productivity (Mann 1973). For example, estimates of giant kelp (*Macrocystis pyrifera*) productivity range from 460 to 3000 g C m^{-2} yr^{-1} (dry weight; Mann 1973; Mann 1982; Coon 1982; Abdullah and Fredriksen 2004) and produce a standing biomass that turns over 6 to 7 times per year (Reed et al. 2008). Bull kelp (*Nereocystis luetkeana*) in British Columbia can assimilate 1400 g C m^{-2} during the sporophyte stage (160 days, Foreman 1984). The magnitude of kelp-derived productivity varies spatially, seasonally, and inter-annually in response to wave disturbance, nutrient and light availability, and ocean climate (Dayton 1985, Graham et al. 2007, Reed et al. 2008, Cavanaugh et al. 2011). *Macrocystis* populations can experience high mortality during El Niño events where there is low nutrient concentration (Dayton et al. 1998, Edwards 2004). Most famously, kelp and other macroalgal populations also vary extensively in response to the trophic cascade involving sea otters, sea urchins, and kelp where sea otters reduce the herbivory on kelp by heavily feeding on sea urchins (Estes and Palmisano 1974).

1.4 Benefits of kelp forests

Kelp forests affect shallow rocky reef ecosystems with their physical structure, biomass, and their associated organisms (Steneck et al. 2002). They provide 3-dimensional structural habitat (Steneck et al. 2002 and references within), reduce local current velocities and dampen waves (Gaylord et al. 2007), and their canopies reduce irradiance at depth thereby affecting understory conditions and species assemblages (Santelices and Ojeda 1984). Kelp is eaten directly by organisms (Bustamante et al. 1995) and provides foraging habitat for kelp associated fishes (Reisewitz et al. 2006, Norderhaug and Christie 2011). Kelp-derived carbon enters coastal food webs as dissolved organic carbon (DOC) released from kelp blades due to microbial activity and direct grazing from herbivores, and as particulate organic matter (POM) as kelp blades senesce and deteriorate (Lucas et al. 1981, Dunton and Schell 1987, Bustamante and Branch 1996). In turn, the proportions of kelp-derived carbon comprising consumers are frequently reported in the range of 40 – 100 % (e.g. Duggins et al. 1989; Bustamante and Branch 1996; Kaehler 2000). Mann (1988) pointed out that kelp may be a stable and continuous source of carbon as they release DOC and POM year round and may be an important subsidy to coastal

systems, particularly during the winter when phytoplankton production is low. Importantly, enhanced contributions of kelp-derived carbon are known to subsidize consumer growth rates (Duggins et al. 1989), with critical implications for population and community dynamics (Bustamante and Branch 1996, Salomon et al. 2008, Markel 2011).

Ecologists are more frequently attempting to integrate landscape and food web approaches that explicitly recognize the importance of the movement of subsidies (nutrients, energy, prey, propagules, genes) among habitats, populations, food webs, and ecosystems (Polis et al. 1997, Massol et al. 2011). Communities that often appear to be too distant or isolated from one another can be interconnected and have significant impacts on each other (Polis and Hurd 1996, Polis et al. 1997). An important realization was the significance of allochthonous subsidies to ecosystem function and stability by affecting consumer-resource interactions, food web dynamics, and biomass across trophic levels (Polis et al. 1997), which has led to the inclusion and quantification of detrital dynamics within food web studies (Moore et al. 2004). Kelp has exceptionally high productivity and high turnover rates, which are responsible for the high production of standing stock biomass and kelp-derived detritus (KDD; suspended and non-suspended; Mann 1973, 1988, Coon 1982, Dayton 1985). Kelp-derived productivity enters local food webs as senescing tissues are eroded by wave action and bacterial decomposition, and subsequently consumed by a wide variety of detritivores and filter-feeders (Hatcher et al. 1977, Mann 1988). Fragments of decaying kelp can litter the ocean floor along coastal areas where they become food for a suite of organisms with a variety of feeding modes (Linley and Newell 1981, Linley et al. 1983). *Macrocystis* can make up a significant portion of California continental shelf kelp drift parcels 9 km away from the kelp source, and in submarine canyons (up to 50 % and 20%, respectively; Harrold et al. 1998). Onshore deposition and surf zone accumulation of kelp can increase productivity of terrestrial and intertidal systems (Polis and Hurd 1996, Orr et al. 2005, Crawley et al. 2009).

1.5 Kelp as a food source

Many studies have compared the relative palatability and nutritional value of kelps, and called into question whether it is even assimilated by consumers (Stuart et al. 1982, Anderson and Velimirov 1982, Duggins and Eckman 1997). Kelp tissues are high in nitrogen content and readily available to consumers and bacteria (Atkinson and Smith 1983, Duggins and Eckman 1997), while other studies state that even aged particles are not a good food source as kelp biomass is assimilated poorly by consumers (Stuart et al. 1982). Fresh *Laminaria groenlandica*, and aged *Agarum f.* and *Alaria m.* are known to support high growth rates for polychaetes and mussels (Duggins and Eckman 1997). Some kelp species have high concentrations of secondary metabolites (Steinberg 1985) which are believed to inhibit growth and gonadal development of consumers (Vadas 1977); however, a review of brown algae phlorotannins (a secondary metabolite) found only 4 of 19 (21%) species on the west coast of North America to have > 2 % of the alga's vegetative dry mass as secondary metabolite (Ragan and Glombitza 1986). A study

by Van Alstyne et al. (1999) found 8 out of 17 (47 %) kelp species had vegetative tissues with levels > 2% by dry mass. Within and among species differences in secondary metabolite concentrations exist, leading to differences in food quality (Duggins and Eckman 1997, Van Alstyne et al. 1999). A species can differ in secondary metabolite concentrations at scales of 1 to 1000 km (Pavia and Aberg 1996) leading to a poor understanding of the patterns of distribution of these compounds (Van Alstyne et al. 1999).

1.6 Oceanographic background

Vancouver Island is situated at the northern end of the California Current Large Marine Ecosystem (Sherman and Alexander 1986). The west coast of Vancouver Island (WCVI) is characterized by rocky reefs and deep fjords along with complex oceanographic processes (Thomson 1981, Thomson et al. 1989) and bottom topography (Allen et al. 2001). The presence of large peninsulas (e.g. Hesquiat and Brooks Peninsulas) is believed to have a significant effect on the water properties and circulation of the local inner shelf (Thomson et al. 1989, Thomson and Ware 2005). The WCVI has a broad continental shelf with a maximum width of 65 km seaward of Juan de Fuca Strait and narrows to a width of less than 5 km near the northern end of the island seaward of Brooks Peninsula (Thomson et al. 1989). The shelf off Barkley Sound, and south to Juan de Fuca Strait, has numerous shallow banks, and a 400 m deep and approximately 10 km wide canyon (Thomson et al. 1989). North of Barkley Sound, the Vancouver Island shelf can be divided at the 100 m depth contour (approximately 10 km from shore) into inner and outer oceanic zones with a moderately steep depth gradient along the 50 m depth contour (Thomson et al. 1989).

The physical oceanography off the WCVI is also quite complex as it is affected by wind, buoyancy fluxes, and tidal currents, which interact with local bottom topography, frictional effects and offshore oceanic processes to create a highly variable system (Thomson et al. 1989). The Davidson Current flows pole-ward over the outer shelf (seaward of the 100 m depth contour) during the winter (Fig. 1.1), but changes direction from north-westward to south-eastward during the spring with the seasonal onset of coastal upwelling (Fig. 1.2; Shanks and Eckert 2005, Thomson et al. 1989). This reverses again in the fall (Thomson et al. 1989). The flow over the inner continental shelf (shoreward of the 100 m depth contour) is dominated by the buoyancy driven Vancouver Island Coastal Current (VICC). This current flows pole-ward year-round but can be reversed in the upper 50 m by strong northwest winds that occur during strong upwelling favourable conditions in summer, or during cold outflow conditions during winter (Thomson et al. 1989). During the summer, a strong southward flowing current (the “Shelf-break Current”) over the outer continental shelf and slope is affected by Coriolis force and turns surface water to the west which leads to upwelling of deep water in this area to replace the displaced surface water (Thomson et al. 1989). Upwelled water can move landward to the edge of the VICC but can only penetrate all the way to the coast during extreme conditions (R.

Thomson, pers. comm.). During the winter, the offshore current flows northward and is called the Davidson Current (Thomson 1981).

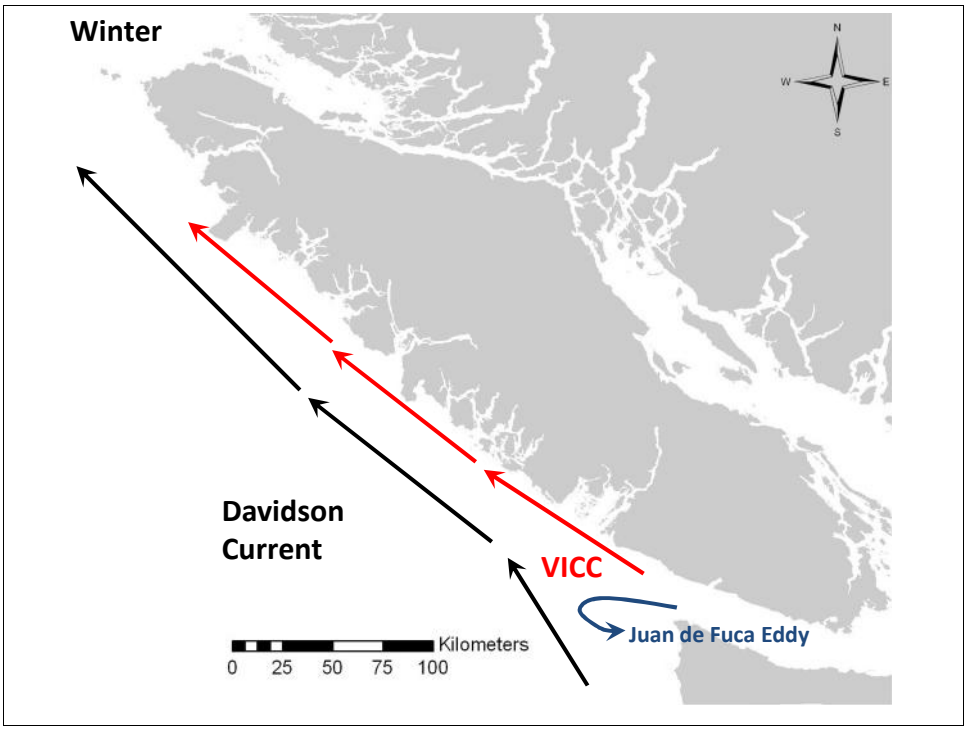

Figure 1.1. Winter currents off the west coast of Vancouver Island. The Vancouver Island Coastal Current is indicated by "VICC". Base map provided by Ed Gregr.

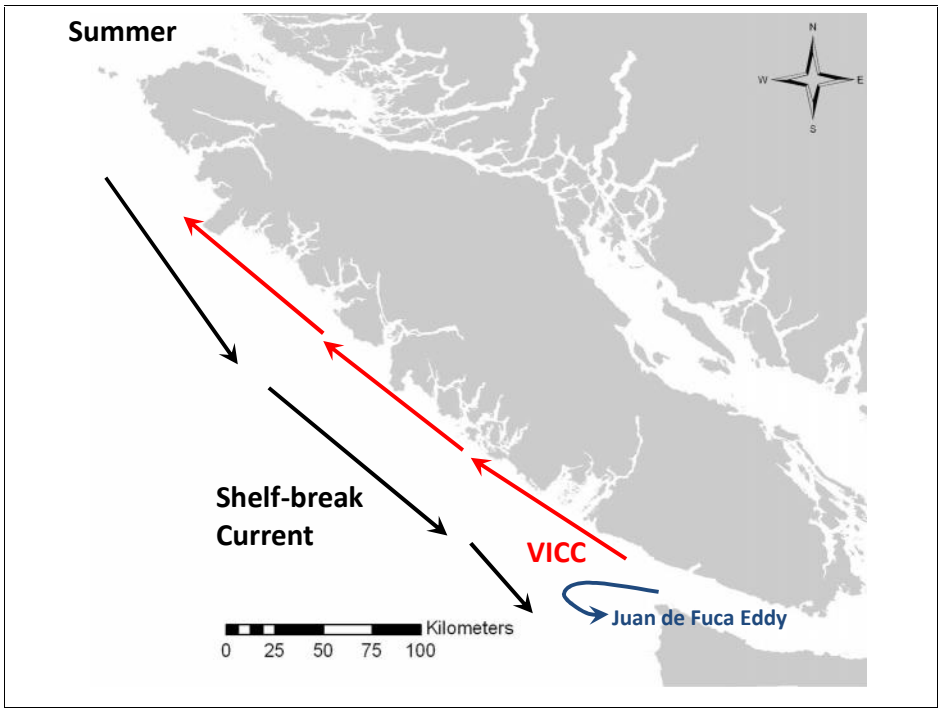

Figure 1.2. Summer currents off the west coast of Vancouver Island. The Vancouver Island Coastal Current is indicated by "VICC". Base map provided by Ed Gregr.

The water properties of the California Undercurrent also have an impact on the regional oceanography of the WCVI (Mackas et al. 1987). This current is characterized as a deep water current (at approximately 300 m depth) that runs along the continental slope from the equator to the Aleutian Islands (Thomson et al. 1989, Allen et al. 2001). This water enters the Juan de Fuca Strait at depth via the Juan de Fuca Canyon (Thomson et al. 1989). Strong tidal mixing brings this nutrient-rich deep water upward into the surface layer where it flows seaward and into the VICC (Mackas et al. 1987, Thomson et al. 1989). This low density outflow from Juan de Fuca Strait spreads out over the shelf before the Coriolis force and the seafloor topography eventually cause the water to narrow into the poleward flowing VICC. This narrowing likely doesn't fully occur until the current is past Barkley Sound. Surface outflow events from Barkley Sound are often seen to disrupt the VICC and extend seaward across the entire shelf (Thomson et al. 1989).

The VICC is continuous along the entire coast to Brooks Peninsula where it abuts the upwelling favourable Shelf-Break Current; however along the coast it can have anomalies (Thomson et al. 1989). During the summer, between Barkley Sound and Juan de Fuca Strait the circulation can lack a clear direction and the VICC can curve seaward off of Barkley Sound. Just north of Kyuquot Sound the continental shelf is narrow (< 5 km wide) and the upwelled water is more easily entrained into the VICC compared to the much wider shelf (~ 65 km) off of Barkley Sound (Thomson et al. 1989, Harris 2001). Another contributing factor to the oceanographic

differences between the north and south coasts of the WCVI is that in the south (off Barkley Sound) the transition zone between the VICC and the seasonal offshore current is pronounced and extensive in the summer (Thomson et al. 1989). Moving to the north, this transition zone narrows as the continental shelf narrows. This could lead to more mixing of nearshore and offshore water masses where the transition zone is less pronounced. This is supported by Forbes and Denman (1991) who report that wind driven upwelling filaments regularly form off Brooks Peninsula and move a significant proportion of the nutrients, phytoplankton and zooplankton off the shelf into the pelagic environment. Flow structure near Brooks Peninsula is complicated and variable (Thomson et al. 1989). It is common to detect the Shelf-break Current moving southward past the end of the peninsula, but it is possible for the VICC to flow north past the peninsula, although it is unclear to what extent it is able to reform (Thomson et al. 1989). During the summer and fall, extensive filaments and/or eddies have been found extending southeastward past Brooks Peninsula (Thomson et al. 1989).

1.7 Freshwater input along the WCVI

Freshwater input from streams and rivers varies spatially and temporally along the WCVI (Dodimead 1984, Leblond et al. 1986). Precipitation run-off from WCVI streams is fed directly into coastal waters and is at a maximum during the fall and winter, and at a minimum during summer (Dodimead 1984, Leblond et al. 1986). During the summer, snowmelt run-off on the mainland on British Columbia is at a maximum and the majority of it enters the Fraser River, and other tributaries (Dodimead 1984, Leblond et al. 1986). This is discharged into the Strait of Georgia where it flows out into the Juan de Fuca Strait. This outflow strengthens the poleward flow and lowers the density nearshore, while enhancing the water column's vertical density gradient (Thomson et al. 1989). The spatially integrated winter run-off rate from streams and rivers along WCVI is approximately half the summer run-off rate from the Fraser River (Leblond et al. 1986).

1.8 Carbon and nitrogen stable isotope background

Carbon and nitrogen stable isotope analysis is a popular tool to trace energy flow within food webs and across ecosystems ($\delta^{13}C$), and assign a trophic level to an individual or groups of species ($\delta^{15}N$; Peterson and Fry 1987, Post 2002). Plant and animal tissues can be analysed to determine the relative abundance of a particular element's stable isotopes (usually carbon and nitrogen). Different groups of organisms have varying stable isotope ratios, which indicate a difference in the relative abundance of heavy vs. light isotopes (e.g., ^{13}C:^{12}C or $\delta^{13}C$ when compared relative to a standard). Higher abundance of the heavy isotope relative to the lighter isotope is referred to as "enriched" and a lower relative abundance is referred to as "depleted". For this reason, food sources that have different isotopic values can be used to determine energy

flow within food webs and to estimate an organism's specific diet (Bustamante and Branch 1996, Post 2002, Fry 2006).

The enriched isotope has an extra neutron which leads to slightly different chemical and physical behaviour relative to its depleted form (Fry 2006). The enriched element forms shorter bond lengths with other elements and molecules, requiring greater energy to make and break during biochemical reactions (Fry 2006). This results in the depleted isotope form reacting faster in kinetic reactions. Additionally, reactions that move both forwards and backwards eventually come to a balanced equilibrium where the enriched isotope will concentrate where bonds are the strongest (Fry 2006). These two principles lead to what is known as fractionation.

The extensive research using stable isotope analysis has revealed large variation in fractionation with trophic level of carbon and nitrogen, especially when considering the tissue analyzed, and feeding mode of different organisms(Vander Zanden and Rasmussen 2001, Post 2002, McCutchan et al. 2003). Lipids are depleted in ^{13}C relative to protein and carbohydrates (DeNiro and Epstein 1977, Focken and Becker 1998) which leads to differences in tissue isotope values. For example, whole animals' $\delta^{13}C$ values are usually lower relative to animals analyzed as simply muscle tissue, which is usually low in lipid (McCutchan et al. 2003). Similarly, carbonate in the shell or bone of aquatic organisms is derived from dissolved inorganic carbon that is generally enriched in ^{13}C relative to its diet (McConnaughey and McRoy 1979). DeNiro and Epstein (1978) concluded that the $\delta^{13}C$ values of whole bodied organisms were enriched over their diet by 0.8 ± 1.1 ‰ with a range of -0.6 ‰ to +2.7 ‰. Since this study there have been a variety of trophic shifts calculated for C ranging from -2.7 to +3.4 ‰ (e.g. Bustamante and Branch 1996, Kaehler et al. 2000, McCutchan et al. 2003). ^{15}N shows a more clear pattern of enrichment with trophic level and assumed to be +2.6 to +3.4 ‰ (DeNiro and Epstein 1981, Minagawa and Wada 1984, Owens 1987). Studies have differed from this with calculations for consumers raised on invertebrates (+1.4 ± 0.20 ‰), high-protein diets (+3.3 ± 0.26 ‰), or algal diets (+2.2 ± 0.30 ‰).

1.9 Kelp and phytoplankton isotope variability

Primary producers' carbon stable isotope values ($\delta^{13}C$) are affected by the interaction of many factors (Rau et al. 1989, 1991, Raven et al. 2002). Dissolved inorganic carbon in seawater is available for cellular uptake as $CO_{2(aq)}$ and HCO_3^- (Raven et al. 2002). These inorganic carbon sources differ in their isotope values ($CO_{2(aq)}$: ~ -10 ‰ and HCO_3^-: ~0 ‰), and are then further depleted during photosynthesis when they are converted to organic carbon (Mook et al. 1974, Francois et al. 1993, Raven et al. 2002). Passive and active diffusion of these two molecules and their relative abundance will have an effect on the primary producer's $\delta^{13}C$ value (Raven et al. 2002). $CO_{2(aq)}$ or HCO_3^- $\delta^{13}C$ has been shown to vary spatially and temporally in estuaries (Fry and Sherr 1984). The potential differential utilization of these carbon sources (Lucas and Berry

1985, Prins and Elzenga 1989) of differing and variable $\delta^{13}C$ values (Mook et al. 1974) could lead to the spatial and seasonal differences in primary producer values.

Nitrogen isotope ratios in marine primary producers reflect the $\delta^{15}N$ of the initial source of inorganic nitrogen, the degree of isotopic fractionation during biotic uptake, and the fraction of the total nutrient supply consumed (Altabet and Francois 1994). The $\delta^{15}N$ of upwelled nitrate, the most abundant nutrient in the North Pacific ecosystem, is approximately 5 to 7 ‰ (Miyake and Wada 1967); the global average of deepwater $\delta^{15}N$ of NO_3^- is ~ 4 – 5 ‰ (Liu and Kaplan 1989, Sigman et al. 1997) and remineralized nitrate is depleted in ^{15}N relative to upwelled nitrate (Altabet 1988). If upwelling is episodic, then temporal decoupling may occur as primary producers consume upwelled NO_3^- leading to an initial decrease in the $\delta^{15}N$ of producers due to isotopic fractionation during uptake followed by an increase in $\delta^{15}N$ as consumption of NO_3^- proceeds to completion (Altabet et al. 1991). Moreover, the degree of fractionation will be lower with more rapid growth and lower nitrogen concentrations, which is perhaps an indication of regional differences with respect to these two factors (Wada and Hattori 1978, York et al. 2007). When nitrate is abundant, primary producers preferentially incorporate ^{14}N (Ostrom et al. 1997). As localized nutrients are consumed due to higher kelp and phytoplankton photosynthetic rates, especially during the summer, ^{15}N discrimination decreases leading to an increase in the kelp $\delta^{15}N$, reflecting the source $\delta^{15}N$ (Altabet and Francois 1994).

In California, $\delta^{13}C$ and $\delta^{15}N$ values of *Macrocystis* have been shown to be more depleted from March to July compared to samples collected from August to December (Foley and Koch 2010). Summer-winter differences in isotope values could be due to highly favourable conditions for growth (i.e. increased solar radiation) during the summer and increased photosynthetic rates as kelp are capable of depleting the available CO_2 at the blade's surface leading to enriched $\delta^{13}C$ values (Farquhar et al. 1989). Foley and Koch (2010) found significantly lower $\delta^{13}C$ values in *Macrocystis* when nitrate concentrations were high and high nitrate concentrations within and nearby the kelp forests during the winter could deplete $\delta^{13}C$ values. Variability in the isotopic composition of biochemical molecules such as fatty acids and lipids has been used as an explanation for $\delta^{13}C$ variation between seasons (Stephenson et al. 1984, Dunton and Schell 1987). Relatively low nitrate concentrations within and nearby the kelp forests during the summer may explain why summer kelp samples have relatively enriched $\delta^{15}N$ values. Localized nutrient drawdown within kelp forests during the summer due to higher kelp and phytoplankton photosynthetic rates results in the decreased discrimination of ^{15}N leading to an increase in the kelp $\delta^{15}N$ (Altabet and Francois 1994). Furthermore, it has been shown that nitrogen content of kelp varies during the growing cycle (Sjøtun et al. 1996) and, as mentioned previously, isotope ratio differences in parts of kelp lamina or at different sampling times could be caused by different biochemical reactions during the growth cycle (Fredriksen 2003).

Time of kelp recruitment, growth chronology, and age composition of the kelp forests sampled (ex. *Macrocystis* and *Pterygophora* are perennials, and *Nereocystis* is an annual) may differ regionally (Stephenson et al. 1984, Simenstad et al. 1993). These factors affect differences

in metabolism and storage of biochemical compounds with differing $\delta^{13}C$ values on shorter time scales (Stephenson et al. 1984, Simenstad et al. 1993). Furthermore, if species distribute $\delta^{13}C$ differently among the blade tissue then the sampling procedure could lead to biases. Benthic marine macrophytes are also subject to varying water movement regimes as a function of the stage in the tidal cycle. This affects the supply of inorganic carbon and nutrients via the thickness of the diffusion boundary layer (Smith and Walker 1980, Wheeler 1980, Raven 1984). For example, macrophytes incorporate depleted carbon and nitrogen preferentially but where water movement and the constant supply of depleted sources is low macrophytes will also be required to incorporate enriched sources as well to maintain metabolic processes, which will lead to macrophyte tissue enrichment. Studies of kelp isotopes in the northeast Pacific Ocean have shown $\delta^{13}C$ to range from -12 to -20.5 ‰ (Page et al. 2008, Foley and Koch 2010). Similarly, $\delta^{15}N$ mean values have been reported to range between 5 and 9 ‰ (Foley and Koch 2010).

Phytoplankton $\delta^{13}C$ values can vary as a result of cell growth rate (Rau et al. 1992), ambient $CO_{2(aq)}$ concentration (Rau et al. 1991), plankton species composition (Fry and Wainright 1991), the type of carboxylation enzyme utilized during photosynthesis (RuBisCO versus PEPCase; Fontugne et al. 1991), irradiance, day length (Thompson and Calvert 1994), active inorganic carbon uptake (Raven et al. 1993), sea temperature (Wong and Sackett 1978) and latitude (Rau et al 1989). Cultures of phytoplankton with silicon added have been shown to grow faster and simultaneously have more diatoms than cultures with no added silicon (Fry and Wainright 1991). The cultures with nutrient added also showed less fractionation. Off the coast of California and Washington, surface POM $\delta^{13}C$ has been correlated to higher chl *a* concentrations. The explanation for this was the greater abundance of diatoms nearshore of an upwelling front, where chl *a* measures were > 5 µg/L, and with smaller phytoplankton (< 5 µm coccooid cyanobacteria and eukaryotic phytoplankton) offshore of the upwelling front where chl *a* concentrations were lower (Sherr et al. 2005, Miller et al. 2008). Dehairs et al. (1997) discuss the importance of extracellular $CO_{2(aq)}$ concentration and the across membrane difference in concentrations. The gradient reflects the cell's demand for carbon and its growth rate which may lead to enriched phytoplankton (Rau et al. 1992, Francois et al. 1993). Other identified factors include carbon fixation pathways and types of enzymes used during carboxylation (Rau et al. 1992, Francois et al. 1993, Dehairs et al. 1997). RuBisCo is an enzyme used in the first step of carbon fixation, and when it is relatively highly active, phytoplankton has been found to be depleted in ^{13}C (Fontugne et al. 1991). When nitrogen uptake by phytoplankton is elevated there is a change in the use of RuBisCo to a β-carboxylase system (Guy et al. 1989, Descolas-Gros and Fontugne 1990). Increased β-carboxylase activity can briefly inhibit photosynthetic carbon fixation to a certain extent (Elrifi and Turpin 1986, Guy et al. 1989). When there is a switch to a β-carboxylase system a different enzyme is used which is called PEPCase. This enzyme uses HCO_3^- substrate that is much more enriched in ^{13}C compared to $CO_{2(aq)}$, as previously mentioned. PEPCase discriminates less against ^{13}C compared to RuBisCo (Guy et al. 1989); however, it has been reported that diatoms use the β-carboxylation enzyme PEPCKase instead of PEPCase (Descolas-Gros and Oriol 1992). PEPCKase is believed to discriminate against ^{13}C

more than PEPCase, and be more similar to RuBisCo (Arnell and O'Leary 1992). Dehairs et al. (1997) used this relationship to postulate that when diatoms are the dominant phytoplankton group, more depleted phytoplankton $\delta^{13}C$ values will occur. In contrast, during another survey, diatoms that were manipulated by doubling the ammonium concentration became more enriched with respect to $\delta^{13}C$ due to an accelerated growth rate (Dehairs et al. 1997, Mengesha 1997). And as Laws (1995) has demonstrated *in situ* and experimentally that even when phytoplankton biomass is low, and absolute carbon fixation is low, cells can become more enriched as a result of high cell turnover and carboxylation rates.

Phytoplankton blooms in their early stages have depleted $\delta^{15}N$ values because phytoplankton utilize $N^{14}O_3^-$ first and then uses $N^{15}O_3^-$ as nitrate becomes limiting (Altabet and Francois 1994). Under nutrient limited conditions, such as the nearing the end of a phytoplankton bloom, all nitrate could be incorporated in its entirety by phytoplankton and have a $\delta^{15}N$ value similar to the source nitrate (Altabet and Francois 1994). Phytoplankton $\delta^{15}N$ has been shown to vary among species and could be a result of cellular nitrogen metabolism, active transport across cell membranes or nitrate reductase activity, among others (Pennock et al. 1996 and references within). There is evidence that phytoplankton $\delta^{15}N$ may vary due to species composition (Montoya and McCarthy 1995) and distinctive cellular mechanisms for nitrogen metabolism and/or differences in intracellular storage of inorganic nitrogen (Dortch 1982, Dortch et al. 1984). In areas of upwelling and inputs of nutrients, there is rapid depletion of surface nutrients and increases in the abundances of ^{13}C and ^{15}N but $\delta^{13}C$ increases are attained faster than $\delta^{15}N$ (Schell et al. 1998).

A study of phytoplankton isotopes in a temperate area off the northeast United States have analyzed net diatoms (> 20 µm) with $\delta^{13}C$ values ranging from -15 to -19 ‰ in warm (~ 20 °C) summer waters (Fry and Wainright 1991). The same study analyzed diatoms from 4 °C water that had $\delta^{13}C$ values ranging from -21 to -22 ‰ and oceanic particulate organic carbon as depleted as -23 ‰. Perry et al. (1999) collected < 28 µm and 28 – 116 µm POM from the continental slope of the WCVI with $\delta^{13}C$ values of -23.6 ± 0.57 (SD) ‰ and -23.0 ±1.07 ‰, respectively. Similar size fractions from the continental shelf had values of -22.1 ± 0.56 ‰ and -19.5 ± 0.83 ‰. In Alaska, incubated phytoplankton cultures that were composed primarily of *Chaetoceros* and *Thalassiosira* had a $\delta^{13}C$ value of -24.0 ± 1.0 ‰ (Duggins et al. 1989).

1.10 Use of stable isotope mixing models

Stable isotope mixing models are used to determine the percent contribution of two or more sources to a mixture , specifically, two or more primary producers to POM or a consumer in the case of our research (Phillips and Gregg 2003, Moore and Semmens 2008, Parnell et al. 2010). Simple, two source mixing models for marine systems typically assume that phytoplankton and a macroalgae, or macroalgae assemblage, are the only primary producers contributing to POM or being incorporated by consumers (Bustamante and Branch 1996,

Kaehler et al. 2000, Miller et al. 2008). Implementation of two source mixing models requires the determination of discrete mean values for the sources, yet these may vary widely among species, seasons and location (Fry and Wainright 1991, Page et al. 2008, Foley and Koch 2010). These models yield results with perceived certainty when this could be far from the truth as there could be high uncertainty and variation in source isotope values used in the model to begin with.

The MixSIR program uses Bayesian analysis of stable isotope mixing models using sampling-importance-resampling (SIR; Rubin 1988, Moore and Semmens 2008) where carbon and nitrogen are equally weighted with respect to how they flow through ecosystems. MixSIR's Bayesian framework determines probability distributions for the proportional contribution of each source to the particular samples (Moore and Semmens 2008). The strength of competing models or parameter values are compared using Bayesian statistics. This allows for the estimation of posterior probability distributions for all proportional contributions via numerical integration. This requires randomly generating projected vectors of proportional source contributions representing all possible outcomes (Moore and Semmens 2008). The probability of each proportional source contribution is then calculated based on the data and prior information (Hilborn and Mangel 1997, Ellison 2004). This method is beneficial as it: includes uncertainty in isotope values when estimating the contribution of sources to an isotope mixture, allows for the characterization of uncertainty in the estimates of source contributions and fractionation based on underlying uncertainty in the mixture and source isotope values, includes uncertainty as a result of there being too many sources for a unique solution, and allows the inclusion of prior knowledge (Moore and Semmens 2008). This modeling technique produces posterior probability distributions for all included sources as well as an estimated distribution median and 95 % confidence interval.

1.11 Thesis goals

In this study, spatial and temporal patterns of suspended kelp-derived carbon off the west coast of Vancouver Island, Canada, were investigated by sampling along a gradient of kelp abundance driven by recovering sea otter populations (Markel 2011). The spatial and temporal variation of phytoplankton and kelp stable isotope values was characterized and used in mixing models to estimate the percent contribution of KDD to particulate organic matter, plankton and benthic organisms. The main questions of forming the basis of this thesis were:

1) Is suspended KDD more abundant in a region where kelp abundance is approximately 20-times higher than a region of low kelp abundance?
2) Does the spatial and temporal distribution of KDD vary in relation to a gradient in sea otter occupancy and kelp abundance?
3) Does KDD contribute proportionately more to the tissues of plankton and benthic organisms near and offshore in a region where kelp abundance is approximately 20-times higher as a result of sea otter recovery?

4) Does the KDD contribution to plankton and benthic organisms vary between summer and winter due to a gradient in sea otter and kelp abundance?

Chapter 2: Quantifying the spatial and temporal variation in phytoplankton and kelp isotopic signatures to estimate the distribution of kelp-derived detritus off the west coast of Vancouver Island

2.1 Introduction

Kelps (Phaeophycea: Order Laminariales) are large fleshy macroalgae that occupy low intertidal and shallow subtidal rocky reef habitats of temperate coastal marine ecosystems (Dayton 1985). Kelp populations affect shallow rocky reef ecosystems by 1) providing 3-dimensional structural habitat (Steneck et al. 2003 and references within); 2) reducing local current velocities and dampening waves (Gaylord et al. 2007); and 3) producing dissolved and particulate matter (Lucas et al. 1981, Dunton and Schell 1987). Kelp-derived productivity contributes substantially to nearshore primary productivity (Mann 1973). For example, estimates of giant kelp (*Macrocystis pyrifera*) productivity range from 460 to 3000 g C m^{-2} yr^{-1} (Mann 1973; Mann 1982; Coon 1982; Abdullah and Fredriksen 2004). Bull kelp (*Nereocystis luetkeana*) in British Columbia can assimilate 1400 g C m^{-2} during the sporophyte stage (160 days, Foreman 1984). Kelp-derived carbon enters coastal food webs as dissolved organic carbon (DOC) released from kelp blades, due to microbial activity and direct grazing from herbivores, or as particulate organic matter (POM) as kelp blades senesce and deteriorate (Bustamante and Branch 1996). In turn, the proportion of kelp-derived carbon assimilated by consumers is frequently reported in the range of 40-100 % (e.g. Duggins et al. 1989, Bustamante and Branch 1996, Salomon et al. 2008). Mann (1988) pointed out that kelp may be a stable and continuous source of carbon as they release DOC and POM year round, particularly in winter months when pelagic production is reduced. Importantly, enhanced contributions of kelp-derived carbon are known to subsidize consumer growth rates (Duggins et al. 1989), with critical implications for population and community dynamics. The magnitude of kelp-derived productivity varies spatially, seasonally, and inter-annually in response to wave disturbance, nutrient and light availability, and ocean climate (Dayton 1985, Graham et al. 2007, Reed et al. 2008, Cavanaugh et al. 2011). Most famously, kelp and other macroalgal populations also vary extensively in response to trophic cascades triggered by sea otters (Estes and Palmisano 1974).

Critically, kelp-derived carbon production is not limited to the footprints of source kelp communities but is widely dispersed by currents and consumers to adjacent ecosystems (Kaehler et al. 2006). Kelp-derived carbon, as drift or suspended POM, can occur at great distances from kelp forests (Hill et al. 2006, Vanderklift and Wernberg 2008, Crawley et al. 2009). For example, kelp-derived carbon has been traced tens of kilometres away from its source (Kaehler et al. 2006, Hill et al. 2006). Ultimately, regional and seasonal patterns of kelp productivity and ocean circulation interact to determine spatial and temporal patterns of the abundance and distribution of suspended KDD. Thus, understanding the spatial and temporal distribution of kelp-derived productivity is necessary for understanding the effects of kelp population dynamics on properties

of coastal marine ecosystems (Duggins et al. 1989, Page et al. 2008, Tallis 2009, Miller et al. 2011).

Determining the spatial distribution of KDD throughout marine ecosystems typically involves 1) assessing the contribution of kelp-derived tissue fragments to samples of suspended POM (e.g. Kaehler et al. 2006); and 2) estimating the proportion of carbon comprising various consumers that was ultimately derived from kelp (or other benthic macroalgae) versus phytoplankton (e.g. Kaehler et al. 2000). Plant and animal tissues can be analysed to determine the relative abundance of a particular element's stable isotopes (usually carbon and nitrogen). Different groups of organisms have varying stable isotope ratios, which indicate a difference in the relative abundance of heavy vs. light isotopes (e.g., ^{13}C:^{12}C or $\delta^{13}C$). Higher relative abundance of ^{13}C is referred to as "enriched" and a lower relative abundance is referred to as "depleted". For this reason, food sources that have different isotopic values can be used to determine energy flow within food webs and to estimate an organism's specific diet (Bustamante and Branch 1996, Post 2002, Fry 2006). Stable isotope mixing models are typically used to estimate the percent contribution of KDD to POM; however, doing so requires determination of discrete POM source stable isotope values, and these may vary widely among species, seasons and location (e.g. Fry and Wainright 1991, Page et al. 2008).

Characterizing the isotopic variability of the sources to POM is key to the reliability of isotope mixing models and their utility for estimating the spatial distribution of KDD. Typically, marine phytoplankton are depleted in ^{13}C relative to brown macroalgae (Dunton and Schell 1987, Duggins et al. 1989). Isolating a nearshore phytoplankton stable isotope value is difficult because seawater collected to obtain phytoplankton samples are likely to also contain KDD and a variety of microzooplankton, bacteria, and flagellates (Koop et al. 1982, Kaehler et al. 2006). To minimize contributions of KDD, POM samples are typically collected well offshore of source kelp forests (e.g. 30 km, Page et al. 2008), and the resulting isotopic values are assumed to be representative of phytoplankton in general. However, offshore phytoplankton is generally more ^{13}C depleted (Gearing et al. 1984, Dehairs et al. 1997, Page et al. 2008), and using these isotope values may result in overestimation of the contribution of KDD to POM (Kaehler et al. 2000, Hill et al. 2006). Other studies have used laboratory cultured phytoplankton to obtain pure phytoplankton samples; however, the isotopic signatures of phytoplankton culture in laboratory environments are poor proxies for those occurring in nature (Duggins et al. 1989).

With respect to kelp, $\delta^{13}C$ values can vary substantially across single blades and at the same position on blades over the course of a year (Stephenson et al. 1984, Simenstad et al. 1993). Highly variable primary producer isotopic values has led some researchers to question their efficacy as sources in linear mixing models, and prefer instead using the isotopic values of primary consumers that integrate stable isotopes over longer time scales and are much less variable (Post 2002). More complex models incorporate uncertainty and calculate ranges of source contributions to a mixture based on stable isotope analyses when the number of sources is too large to permit a unique solution (Phillips and Gregg 2003, Moore and Semmens 2008,

Parnell et al. 2010). Regardless of model choice, establishing accurate stable isotope values of sources is critical to quantifying model uncertainty.

In this study, spatial and temporal patterns of suspended kelp-derived carbon off the west coast of Vancouver Island, Canada, were investigated by sampling along a gradient of kelp abundance driven by recovering sea otter populations (Markel 2011). The main questions of this chapter were twofold. First, is suspended KDD more abundant in a region where kelp abundance is approximately 20-times higher (Markel 2011) as a result of sea otter populations that dramatically reduce the abundance of grazing sea urchins. Second, does the spatial and temporal distribution of KDD vary in relation to this sea otter and kelp abundance gradient? Kelp and phytoplankton isotopic variability were investigated to quantify the percent relative contribution of KDD to POM. It was predicted that the region with much higher kelp productivity would have more KDD in the water column nearshore and offshore, during both summer and winter months.

2.2 Materials & Methods

2.2.1 Study system

Oceanographic context - This research was conducted on the West Coast of Vancouver Island (WCVI), British Columbia, Canada (Fig. 2.1), situated at the northern end of the California Current Large Marine Ecosystem (Sherman and Alexander 1986). This coastline is characterized by complex oceanographic processes. Seawater over the outer shelf (seaward of the 100 m depth contour) flows pole-ward during the winter but changes direction from north-westward to south-eastward during the spring with the seasonal onset of coastal upwelling (Shanks and Eckert 2005, Thomson et al. 1989). The flow over the inner continental shelf (shoreward of the 100 m depth contour) is dominated by the buoyancy driven Vancouver Island Coastal Current (VICC). This current flows pole-ward year round but can be reversed in the upper 50 m by strong northwest winds that occur during strong upwelling favourable conditions in summer or during cold outflow conditions during winter (Thomson et al. 1989). Coastal upwelling brings cold and nutrient rich deep water to the surface where it supplies primary producers with essential nutrients for photosynthesis (Dugdale 1985). Most of the ocean-based photosynthesis occurs along the coastal margins, which occupy only 0.1 % of the total ocean area, and has been estimated to support phytoplankton productivity in upwelling regions ranging from 200 to 973 g C m^{-2} yr^{-1} (Pauly and Christensen 1995, Hahm and Kim 2001). Phytoplankton concentrations along the WCVI reach a maximum during late spring to mid-summer when solar radiation is high and nutrients are readily available from winter mixing of the water column and upwelling (Parsons and Lalli 1988, Whitney et al. 1998).

Figure 2.1. Sampling regions along the west coast of Vancouver Island (WCVI). The red lines represent the summer transects (0 – 30 km). During the winter, sampling was only to 10 km from the kelp forest in the otter-present and otter-absent regions, and only at 4 and 8 km in the otter-intermediate region. Sampling transects indicated by T1, T2, etc.

Ecological context.- Sea otters are voracious consumers and exert strong top-down control of many benthic invertebrates, most notably sea urchins (Estes and Palmisano 1974). In the absence of sea otters, hyper-abundant urchin populations dramatically reduce macroalgal populations and result in widespread urchin "barrens" (Pearse and Hines 1979). In the presence of sea otters and absence of sea urchins, expansive kelp forests support diverse and productive ecosystems (Estes and Palmisano 1974, Dayton 1985, Duggins et al. 1989, Steneck et al. 2002). As a result of the North Pacific maritime fur trade during the 18th and 19th centuries, sea otters were commercially and ecologically extinct along the coast of British Columbia by the late 19th century and were extirpated from the coast by 1929 (Cowan and Guiguet 1960, Kenyon 1969). However, between 1969 and 1972 eighty-nine animals were reintroduced to the northwest coast of Vancouver Island from western Alaska (Bigg and MacAskie 1978). Subsequently, this population has increased rapidly and expanded its range north and southwards along the WCVI (Nichol et al. 2005, 2009). At the time of sampling, sea otters were distributed from the northern tip of Vancouver Island southward to an area just south of Clayoquot Sound (Fig. 2.1) and have

caused an increase in kelp forest size and standing stock biomass along this coastline (Markel 2011, Watson and Estes 2011). There are 27 kelp species in the coastal waters of British Columbia (Lucas et al. 2007) with most flourishing along the WCVI (including *Costaria costata*, *Desmerestia* spp., *Eisenia arborea*, *Laminaria setchellii*, *Hedophyllum sessile* and *Egregia menziesii*; Druehl 2000, Watson and Estes 2011). The three dominant species that this study focused on include two species of perennial kelp, giant kelp *Macrocystis pyrifera*, and old growth kelp *Pterygophora californica*, and one annual species, bull kelp *Nereocystis luetkeana* as they were deemed to have the greatest abundance during recent surveys (Martone and Markel, in prep).

2.2.2 Experimental Design

Three research cruises were conducted off the west coast of Vancouver Island, British Columbia between July 2009 and July 2010. The study region includes three large Sounds that occur along this coastline and are characterized by large differences in kelp biomass corresponding to sea otter occupation time (Markel 2011, Watson and Estes 2011). A 'space-for-time substitution' approach (Pickett 1989) was used to take advantage of the natural experiment created by sea otter reintroduction and range expansion on the WCVI. Kyuquot Sound, in the north (nearest to where sea otters were first reintroduced and re-established), has the highest kelp biomass and is hereafter referred to as 'otter-present'. Clayoquot Sound is hereafter referred to as 'otter-intermediate' and Barkley Sound with the least kelp biomass (Markel 2011, Watson and Estes 2011) is referred to as 'otter-absent'. The otter-present and otter-absent regions were approximately 170 km apart. Two transects (approximately 10 to 12 km apart) originated within kelp forests at the mouth of each sound and ran perpendicular to the coastline with sampling at 0, 0.5, 1, 2, 4, 10 and 30 km from the kelp forest. During the winter, due to inclement weather, samples were collected to just 10 km from the kelp forests and some stations did not have the whole suite of samples collected. In the 'otter-absent' and 'otter-present' regions summer sampling occurred between July 18th and 23rd, 2009; summer sampling off the otter-intermediate region was conducted between July 27th and July 28th, 2010. Winter sampling took place off all three regions between January 23rd and 31st, 2010. Transect 1 and 2 (T1 and T2) are within the otter-present region, transect 5 and 6 (T5 and T6) within the otter-intermediate region, and transect 3 and 4 (T3 and T4) are within the otter-absent region.

2.2.3 Physical, chemical, and biological measurements

Sea Bird (SBE) CTDs (SBE 19 during January 2010 and SBE 25 during July 2010) were deployed along transects to measure conductivity, temperature and pressure measurements were obtained. Seawater for nutrient concentration (NO_3^-, PO_4^- and SiO_2) analyses was collected from the surface at each station using a bucket (except transect 4 off the otter-absent region in July 2009) and 2-4 m above the sea floor using Niskin bottles at 1, 10 and 30 km stations. Particle

free water was obtained using a with 0.45 µm Supor® membrane filters and analyzed on a Bran & Luebbe Auto-Analyzer 3 using air-segmented continuous-flow analysis. Dissolved nutrient data are in Appendix A2.

Seawater for chl *a* concentration analyses was also collected from the surface with a bucket and filtered in replicates: > 20 µm and > 0.7 µm (hereafter referred to as "total chl *a*"). Chl *a* was extracted in 10 ml of 90 % acetone in darkness at -20°C for 48 to 72 hours. Pigment concentration was measured on a Turner Designs TD-700 fluorometer before and after acidification (chl *a* data are available in Appendix A1).

2.2.4 Size-fractionated POM stable isotopes

To collect surface water size-fractionated particulate organic matter (POM), water was collected with a bucket at each station and then fractionated into 0.7-20 µm, 20-63 µm, and > 0.7 µm (hereafter referred to as "total POM") fractions using 0.7 µm Whatman GF/F and 20 µm MAGNA nylon filters.

All samples were filtered in the field and immediately frozen. In the laboratory, all total POM filters were analyzed under a dissecting microscope at 2x magnification and all zooplankton and other visible contaminants were removed by hand. Organic matter on the 20 µm MAGNA nylon filters was transferred to Whatman GF/F filters in the laboratory. This was done by moistening the 20 µm filter with distilled water and then scraping loose the organic matter with a sterilized metal spatula followed by rinsing this filter onto a GF/F filter. All filters were dried in a Fisher Scientific oven at 50ºC for at least 24 hours and packaged into 8x11 mm tin capsules for stable isotope analysis.

All isotope analyses were conducted at IsoEnvironmental in Grahamstown, South Africa. Abundances of naturally occurring carbon and nitrogen stable isotopes were determined on either a Europa Scientific Integra IRMS or Europa Scientific 20-20 IRMS linked to an ANCA SL Elemental Analyser. Beet sugar and ammonium sulphate were used as internal standards, calibrated against several International Atomic Energy Agency (IAEA) reference materials. Results are expressed in the standard delta notation, as $X = [(R_{sample} / R_{standard}) - 1] \times 1000$, where X = element in question and R = ratio of the heavy over the light isotope. Repeated analyses of homogeneous material yielded a standard deviation of 0.2 ‰.

2.2.5 Macroalgae stable isotopes

Fresh samples of the kelps *Nereocystis luetkeana* and *Macrocystis pyrifera* were collected at stations within kelp forests (0 km stations) of each transect during summer (July) and winter (January) and immediately frozen in a -20 °C. In the laboratory, kelp samples were thawed, contaminants removed, rinsed with distilled water, and dried for at least 24 hours at 50ºC. Dried

samples were ground into a fine powder using a mortar and pestle or digital amalgamator, and packaged in tin capsules for carbon and nitrogen stable isotope analysis. Additional samples of fresh kelp that overlapped with our sampling area were available from Gerald Singh (Department of Zoology, UBC). However, this additional summer and winter kelp sampling occurred in June – August 2009 and January – March 2010, respectively. For these additional samples, summer and winter otter-present region samples were collected during August and March, respectively. Otter-intermediate region summer samples were collected during July. Otter-absent region summer and winter region samples were collected during June and February, respectively.

Drift kelp of the same species was sampled opportunistically along the offshore transects. The free-floating kelp that was no longer attached to the bottom via its holdfast was considered to be decaying. Various decomposed green (Chlorophyta), brown (Ochrophyta), and red (Rhodophyta) macroalgae were collected using a dredge. These samples were frozen at sea and later thawed, identified to the lowest possible taxonomic level, and dried and ground for carbon and nitrogen stable isotope analysis. These algae were not used in the mixing model due to the small sample size (*Macrocystis*, n=3 and *Nereocystis*, n=6) and poor spatial coverage and therefore will not be discussed further.

2.2.6 Data analyses

Phytoplankton stable isotope value determination: Principal component analysis (PCA) was used to characterize spatial and seasonal variability in phytoplankton $\delta^{13}C$ and $\delta^{15}N$ by relating these values to oceanographic and biological parameters, including:: total chl *a* (µg/L), > 20 µm chl *a* (µg/L), nitrate (µmol/L), silica (µmol/L), phosphate (µmol/L), sea surface temperature (°C), ocean depth (m). Sea surface temperatures were obtained from the Ocean Watch live access server's "Multiple-satellite blended products" dataset (http://las.pfeg.noaa.gov/oceanWatch/oceanwatch.php#). Sea surface temperature data were extracted with an "eight day composite" prior to sampling dates at each station. A variable was said to load on a particular component if the factor loading was 0.40 or greater for that component, and was less than 0.40 for the other component (Stevens 1986). Components were deemed meaningful if the eigenvalues were greater than 1 and the results were confirmed by a scree test (Abdi and Williams 2010). Each station's suite of sampled parameters was also compared to what is known in the literature with respect to the relationships among these parameters and their effects on isotope variability. Together, I used this information to determine seasonally unique carbon and nitrogen isotope values for three size fractions (0.7 – 20 µm pico-/nano-plankton, 20 – 63 µm and total phytoplankton) of "blooming" and "non-blooming" phytoplankton to use in multiple source stable isotope mixing models.

Kelp stable isotope values: Because kelp was not sampled in the otter-intermediate region during the winter, I estimated *Macrocystis* and *Nereocystis* isotope values for this region by averaging the difference between summer and winter isotope values for these species in the otter-present and otter-absent regions. These average differences were then added to the otter-

intermediate summer isotope values. Otter-intermediate winter kelp standard deviations were estimated by averaging the otter-present and absent standard deviations for their respective kelp species. Winter standard deviations were used in this manner to capture any potential greater isotope value variability during the winter. Abundance estimates of dominant kelp species were obtained from (Martone and Markel, in prep) and their relative percent contributions (Table 2.3) were multiplied by their respective regional stable isotope values and standard deviation. A region's dominant kelp species proportional contributions were then added back together to give a single kelp value to include in the mixing model. Individual kelp species were not used in the mixing model as to estimate the contribution of kelp, in general, and for presentation purposes. Data was not available for winter *Pterygophora* isotope values. On average, *Macrocystis* and *Nereocystis* were depleted by 0.23 ‰ in ^{13}C during the winter and had an average standard deviation of 1.66. *Macrocystis* and *Nereocystis* $\delta^{15}N$ values were on average depleted by 1.73 ‰ during the winter and had an average standard deviation of 1.29. These values were then applied to the summer otter-intermediate *Pterygophora* data. Where assumptions were met, analysis of variance (ANOVA), two-way ANOVA, and t-tests were used to test for within and among region differences in kelp species' stable isotope values. In cases of non-normality or heterogeneous variance, pair-wise t-tests with Bonferroni correction, or Mann Whitney U tests were carried out. Only statistically significant differences and trends will be presented. All statistical analyses were performed in R (R Core Development Team 2011).

MixSIR: The isotope mixing model program MixSIR (version 1.0, Moore and Semmens 2008) was used to estimate the percent KDD contribution to POM samples. MixSIR is a graphical user interface that performs Bayesian analysis of stable isotope mixing models using Hilborn sampling-importance-resampling (SIR) algorithm (Rubin 1988). The benefits of a Bayesian approach to stable isotope mixing models include: 1) accounting for uncertainty in source stable isotope values and fractionation, 2) accounting for uncertainty in the estimates of source contributions as there is underlying uncertainty in the mixture and source isotope values, 3) determining a unique solution when more than two sources are present (Semmens and Moore 2008, Moore and Semmens 2008). Outputs of the program include a median percent contribution of all sources included and their respective 95 % confidence intervals. The degree of overlap between confidence intervals defines the probability that two estimates are the same. Assuming there was no isotope fractionation during the formation of kelp- and phytoplankton-derived particulates, source isotope fractionation and fractionation standard deviations were set to '0'.

2.3 Results

2.3.1 Surface nitrate and chlorophyll

Summer total and >20 µm chl a concentration: The mean summer total (>0.7 µm) chl *a* concentration (± SD) off the otter-present region was 7.16 ± 2.54 µg/L and ranged widely from 1.66 to 10.71 µg/L. The first 4 kilometers sampled along both transects were quite similar in chl

a concentration; beyond this station transect 1 had a steady and moderately high (6.5 – 8 µg/L) chl *a* concentration moving offshore, while transect 2 dropped drastically to less than 3 µg/L at 10 and 30 km offshore (Fig. 2.2 A).The mean summer > 20 µm chl *a* concentration of the otter-present region was 6.40 ± 2.73 µg/L with a range of 0.92 to 10.25 µg/L.

The mean summer total chl *a* concentration off the otter-intermediate region was 5.48 ± 1.98 µg/L which ranged from 3.14 to 9.29 µg/L. Transect 5 concentration was moderate overall and did not decrease with distance from shore while remaining at a concentration near 5 µg/L. Transect 6 chl *a* concentration was approximately twice as high as transect 5 from 0 to 0.5 km offshore. Both transects were similar in concentration at nearly all distances from shore, except within the kelp forest itself (0 km), where transect 6 had nearly twice the chl *a* concentration (Fig. 2.2 C).

The mean summer total chl *a* concentration for the otter absent region was 8.8 ± 4.35 µg/L with a range of 3.01 to 14.35 µg/L. The mean summer > 20 µm chl *a* concentration of the otter-absent region was 7.96 ± 4.57 µg/L with a range of 2.45 to 15.13 µg/L. Both transects were similar in concentration at 0 and 0.5 km from shore, however at 1 km transect 3 chl *a* concentration was over twice as high as transect 4. At 4 km from shore transect 4 chl *a* concentration begins to decrease compared to transect 3 and reaches a minimum at 10 km with a concentration of 2.45 µg/L and stayed relatively low at 30 km from shore. At these distances transect 3 > 20 µm chl *a* concentration stayed relatively high (>11 µg/L; Fig. 2.2 C).

Overall, the > 20 µm fraction made up the majority of the chl *a*, relative to the 0.7 – 20 µm fraction, present at a mean of 88.9 ± 0.1 % of the total chl a concentration with a range of 55.8 – 100 %.

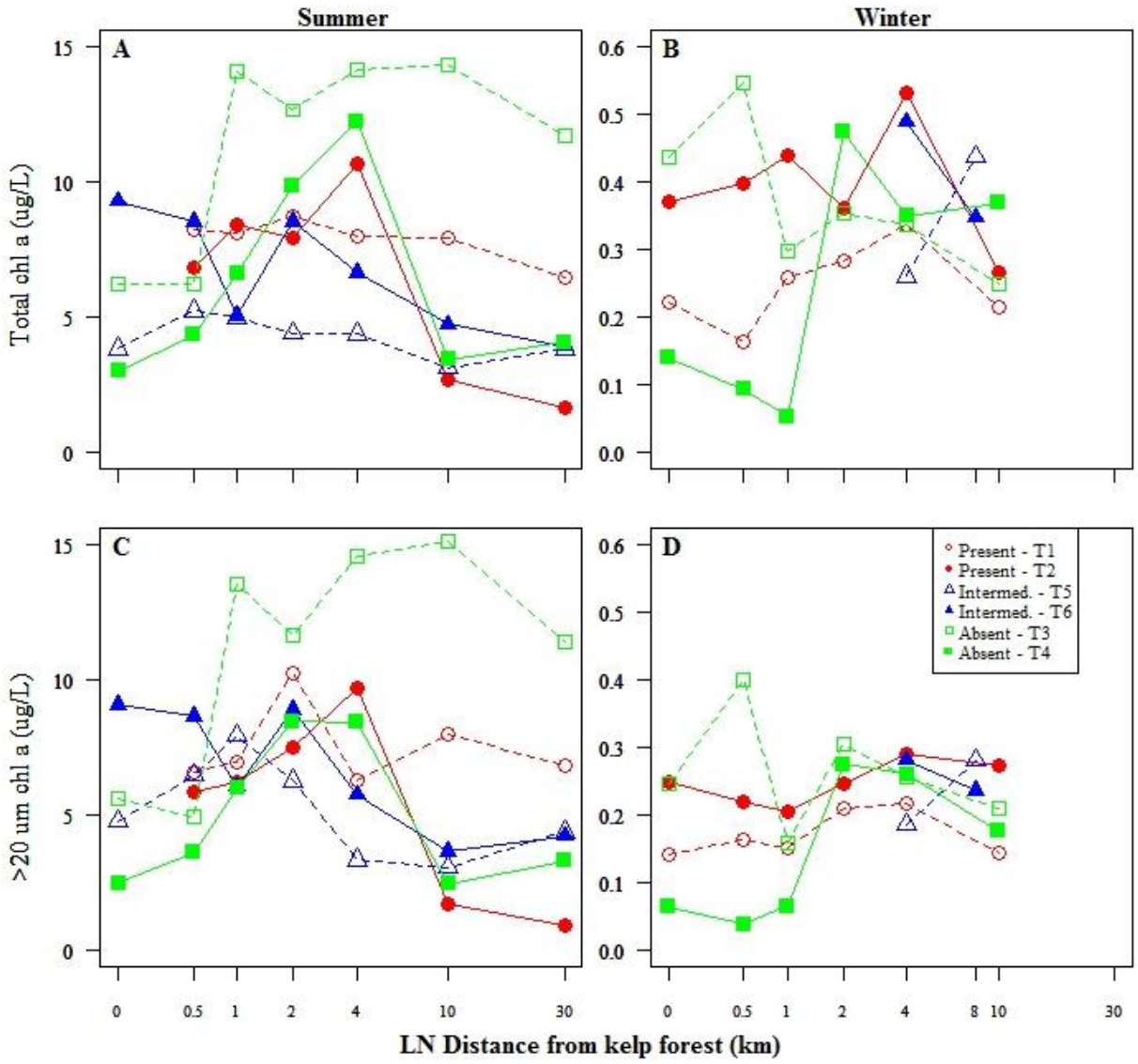

Figure 2.2. Summer and winter total (>0.7 µm) and > 20 µm chl *a* concentration (µg/L) with natural log distance from the kelp forest for transects from all sea otter abundance gradient regions. Each point represents a single sample (n=1). For presentation purposes distance is in natural log scale.

Winter total and > 20 µm chl a concentration: The mean winter total chl *a* concentration off the otter-present region was 0.25 ± 0.06 µg/L with a range of 0.16 to 0.53 µg/L (Fig. 2.2 B, Table A1). Chl *a* concentration along both transects remained relatively constant with distance from the kelp forest (Fig. 2.2 B).

The mean winter total chl *a* concentration off the otter-intermediate region was 0.38 ± 0.1 µg/L with a range of 0.26 to 0.49 µg/L (Fig. 2.2 B, Table A1). The mean winter > 20 µm chl *a* concentration off the otter-intermediate region was 0.25 ± 0.05 µg/L with a range of 0.19 to 0.28 µg/L (Fig. 2.2 D).

The mean winter total chl *a* concentration off of the otter-absent region was 0.31 ± 0.15 µg/L with a range of 0.05 to 0.55 µg/L. Transect 3 decreases moderately with distance from the kelp forest while transect 4 has concentrations < 0.2 µg/L from 0 to 1 km from shore and then peaks at 2 km and remains relatively high (> 0.35 µg/L) to 10 km (Fig 2.2 B). The mean winter > 20 µm chl *a* concentration off Barkley Sound was 0.20 ± 0.11 µg/L with a range of 0.04 to 0.40 µg/L. Both transects follow a similar pattern as the total chl *a* size fraction (Fig. 2.2 D).

Overall, the chl *a* concentration was fairly constant moving offshore for both size fractions in all regions. The > 20 µm fraction made up the majority (on average, 66.9 ± 16 %) of the chl *a* present, and ranged from 41 – 100 %. The summer concentrations in all regions and at all distances from the kelp forest are generally an order of magnitude greater than during the winter.

Nitrate concentration: Summer nitrate concentrations ranged from 0 to 9.5 µmol/L and were highly variable among regions. Generally, nutrient concentrations decreased with increasing distance from the kelp forests; however, along transect 3 in the otter-absent region there was virtually no detectable nitrate at any stations (Fig. 2.3 A). Winter nitrate concentration ranged from 8.8 to 13.9 µmol/L. Both transects in the otter-present region and transect 3 in the otter-absent region had relatively constant concentrations with distance from kelp forests. Transect 4 in the otter-absent region had a relatively lower concentration (8.8 to 9.7 µmol/L) from 0 to 1 km from the kelp forest but then increased from 2 to 4 km where it peaked at 13.7 µmol/L.

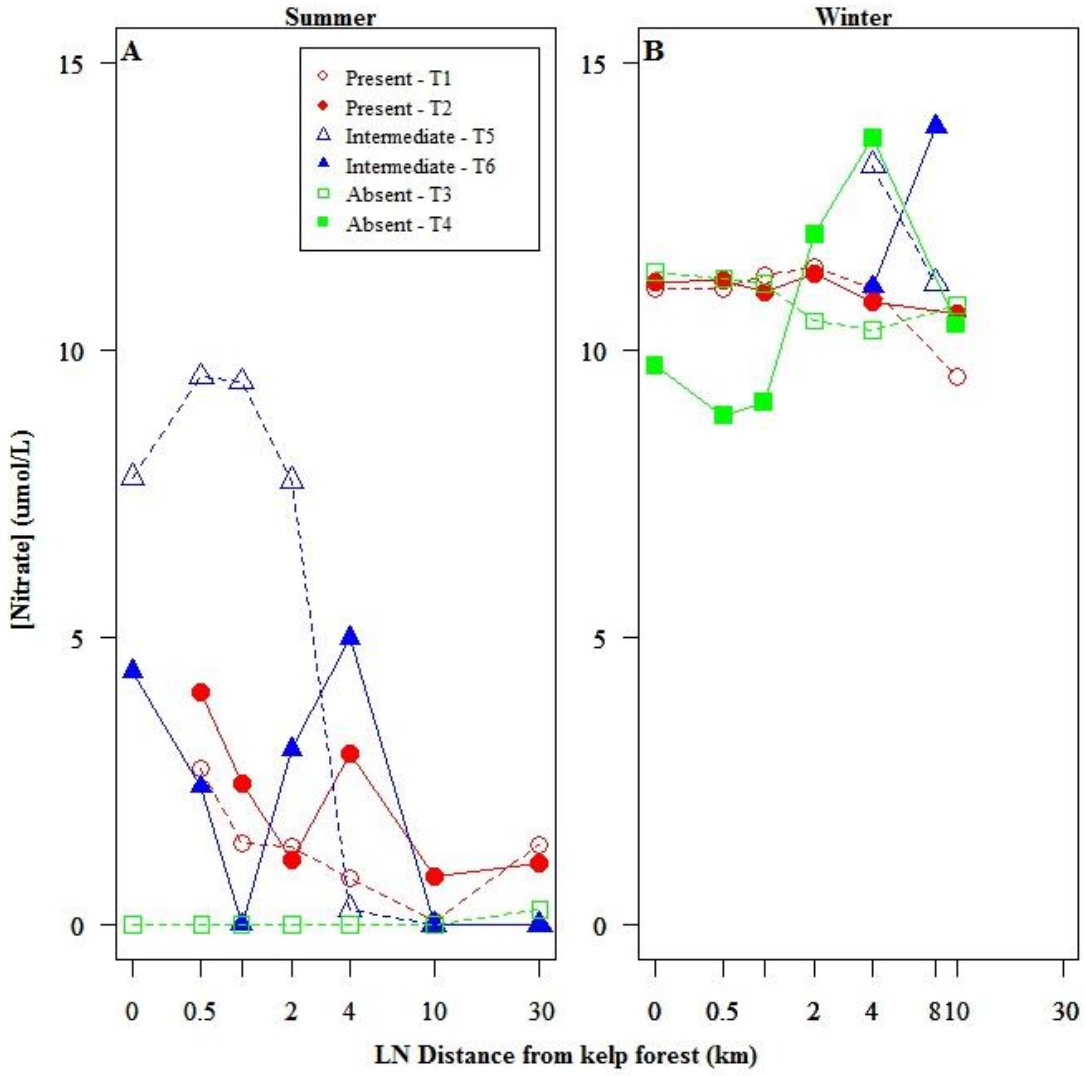

Figure 2.3. Summer (panel A) and winter (panel B) nitrate concentration (µmol/L) with natural log distance from the kelp forest for transects from all sea otter abundance gradient regions. There is no data available for summer "Absent - T4". For presentation purposes distance is in natural log scale.

2.3.2 Kelp carbon stable isotope values

***Summer*:** $\delta^{13}C$ values of kelp varied among species, regions, and seasons (Fig. 2.4, Table 2.1). Kelp species (two-factor ANOVA, $F_2 = 58.4$, $p < 0.001$) and region ($F_2 = 9.7$, $p < 0.001$) were significant while the interaction was not, which indicates that overall kelp species had different $\delta^{13}C$ values and there were regional differences when species were grouped. Tukey's test revealed *Macrocystis* was more enriched (3.72 ‰) than *Nereocystis* in the otter-present ($p < 0.001$) and the otter-absent (3.89 ‰, $p < 0.001$) regions, and *Pterygophora* ($p < 0.001$, for both regions; 5.25 and 5.07 ‰, respectively). No across region differences existed for any of the three species.

***Winter*:** During the winter, *Macrocystis* from the otter-absent region was more significantly enriched than *Nereocystis* ($W = 276$, $p = 0.04$). No otter-intermediate region samples were collected and no *Pterygophora* samples were collected in any region (Fig. 2.4).

***Summer-Winter Comparison*:** During summer *Macrocystis* from the otter-present region was significantly enriched in ^{13}C than during the winter by 2.2 ‰ ($W = 379$, $p < 0.001$). The otter-absent region summer *Nereocystis* $\delta^{13}C$ was more enriched by 2.16 ‰ over winter ($W = 99$, $p < 0.001$).

2.3.3 Kelp nitrogen stable isotope values

The stable isotope values of kelp varied widely between regions and seasons. Refer to Table 2.1 for complete summary of regional kelp isotope values and kelp isotope values used in mixing model analyses are in Table 2.3.

***Summer*:** Kelp $\delta^{15}N$ values differed within and among regions (Fig. 2.4). In the otter-present region *Macrocystis* and *Nereocystis* were significantly more enriched than *Pterygophora* ($p < 0.001$, in both cases). In the otter-intermediate region, *Macrocystis* was significantly more enriched than *Pterygophora* by 1.22 ‰ ($p = 0.03$). In the otter-absent region, *Macrocystis* was more enriched than *Nereocystis* ($p = 0.045$) and *Pterygophora* ($p < 0.001$). $\delta^{15}N$ values of neither *Macrocystis* or *Pterygophora* differed between region; however, *Nereocystis* $\delta^{15}N$ values were significantly enriched in the otter-present region relative to the otter-intermediate region ($p = 0.007$).

***Winter*:** *Macrocystis* $\delta^{15}N$ values in the otter-present regions were significantly more enriched compared to the otter-absent region by 2.45 ‰ ($t = -4.4$, $df = 37.6$, $p < 0.001$). *Macrocystis* $\delta^{15}N$ values in the otter-present region were also significantly enriched relative to *Nereocystis* within this region by 1.67 ‰ ($t = 2.7$, $df = 40.8$, $p = 0.009$). Again, the otter-intermediate region macroalgae was not collected and the same procedure as above was followed to obtain appropriate mixing model values (Fig. 2.4, Table 2.1).

Summer-Winter Comparison: Summer *Nereocystis* $\delta^{15}N$ values in the otter-present region were more enriched ($t = 4.4$, $df = 32.3$, $p < 0.001$) than winter *Nereocystis* by 2.12 ‰. Summer *Macrocystis* $\delta^{15}N$ values in the otter-absent region were more enriched ($W = 543$, $p < 0.001$) by 3.12 ‰ over winter *Macrocystis*. Summer *Nereocystis* $\delta^{15}N$ values in the otter-absent region were more enriched ($W = 445$, $p < 0.001$) than winter *Nereocystis* by 1.48‰.

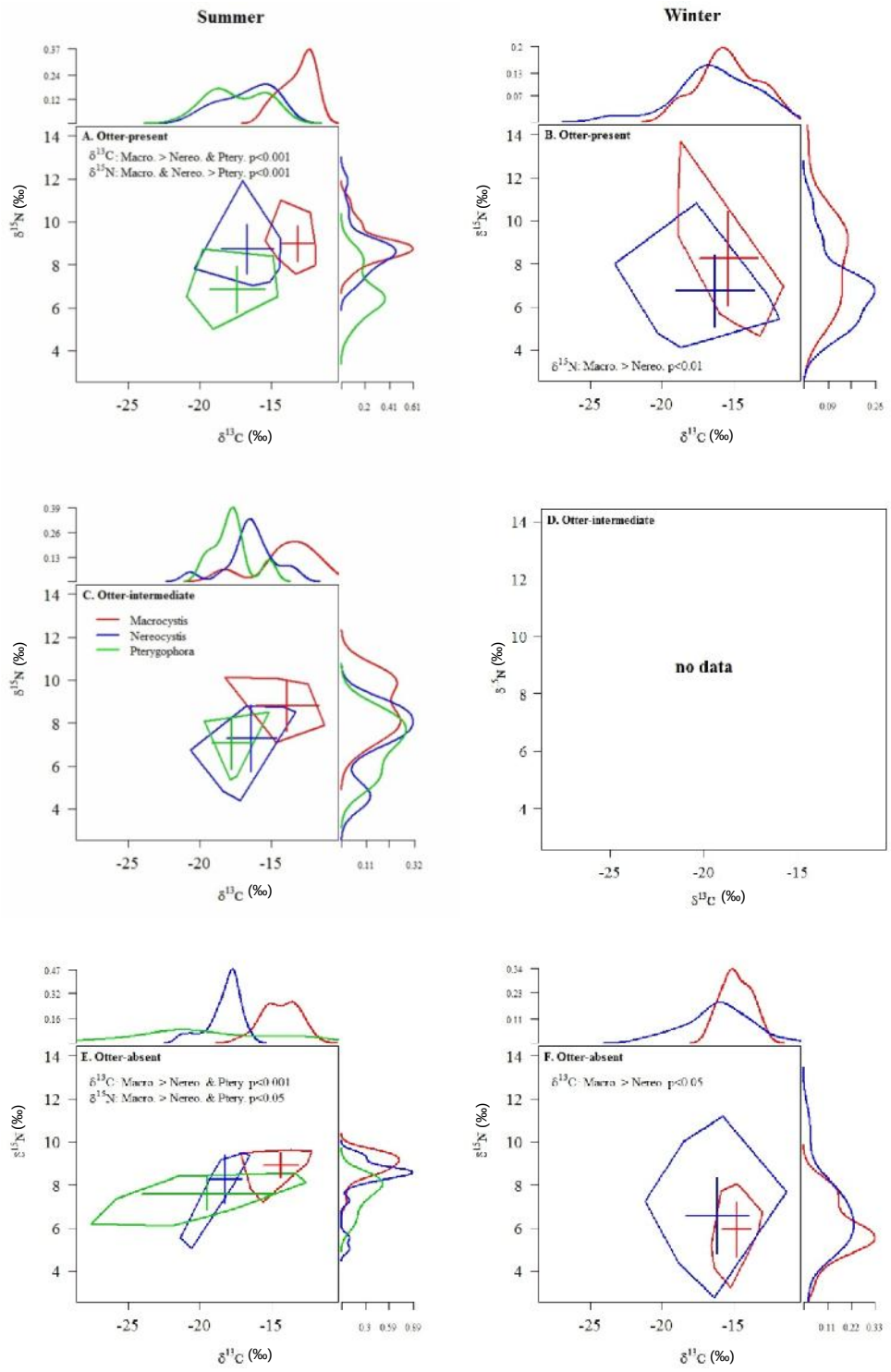

Figure 2.4. Summer and winter *Macrocystis*, *Nereocystis* and *Pterygophora* $\delta^{13}C$ and $\delta^{15}N$ values (‰ +/- SD) in convex hull plots for otter abundance gradient regions. Horizontal and vertical lines represent one standard deviation and intersect at the mean. Species-specific distributions are plotted in the margins. No data is available for *Pterygophora* during the winter in all regions and there is no winter otter-intermediate region data. Marginal distributions are relative frequencies.

2.3.4 Kelp C:N ratios

Summer: Kelp C:N ratios differed within and among regions (Fig. A2). Kelp species (two-factor ANOVA, $F_2 = 24.1$, $p < 0.001$), region ($F_2 = 107.2$, $p < 0.001$) and the species-region interaction ($F_3 = 33.6$, $p < 0.001$) were all significant indicating that species of kelp had differing C:N ratios, regions differed when species were grouped, and there were within species differences among regions. Tukey's test showed the otter-present region *Nereocystis* had lower C:N ratios than *Pterygophora* ($p = 0.03$). In the otter-absent region *Macrocystis* C:N ratios were higher than *Nereocystis* ($p < 0.001$). C:N ratios in the otter-absent region were higher than *Macrocystis* in either the otter-present ($p < 0.001$) or otter-intermediate regions ($p < 0.001$). The elevated mean C:N ratio of *Macrocystis* and *Nereocystis* in the otter-absent region was likely due to samples collected from Diana Island which were near a freshwater stream (Gerald Singh, pers. comm.), which is known to lead to relatively high C:N ratios.

Winter: In contrast to summer, C:N ratios of *Macrocystis* and *Nereocystis* did not differ within or between regions (Fig. A2).

Summer-Winter Comparison*:* The otter-absent region summer mean *Macrocystis* C:N ratio was greater during the winter ($W = 73$, $p = 0.002$) by 26.85. Again, this difference was driven by only three summer *Macrocystis* C:N samples from this region, all from Diana Island. During the winter there were many more *Macrocystis* C:N samples available from different locations that had much lower C:N ratios.

A.	**Otter-present**				**Otter-intermediate**				**Otter-absent**			
Season	**Summer**		**Winter**		**Summer**		**Winter**		**Summer**		**Winter**	
Species	**Mean $\delta^{13}C$ ± SD (‰)**	**Sample size (n)**	**Mean $\delta^{13}C$ ± SD (‰)**	**Sample size (n)**	**Mean $\delta^{13}C$ ± SD (‰)**	**Sample size (n)**	**Mean $\delta^{13}C$ ± SD (‰)**	**Sample size (n)**	**Mean $\delta^{13}C$ ± SD (‰)**	**Sample size (n)**	**Mean $\delta^{13}C$ ± SD (‰)**	**Sample size (n)**
Macrocystis pyrifera	-13.17 ± 1.12	20	-15.37 ± 2.06	23	-13.94 ± 2.25	7	nd	nd	-14.42 ± 1.26	26	-14.95 ± 1.06	21
Nereocystis luetkeana	-16.89 ± 1.88	19	-16.32 ± 2.78	19	-16.41 ± 1.78	14	nd	nd	-18.31 ± 1.17	21	-16.15 ± 2.27	26
Pterygophora californica	-17.42 ± 1.97	18	nd	nd	-17.77 ± 1.42	7	nd	nd	-19.49 ± 4.63	15	nd	nd
B.	**Mean $\delta^{15}N$ ± SD (‰)**	**Sample size (n)**	**Mean $\delta^{15}N$ ± SD (‰)**	**Sample size (n)**	**Mean $\delta^{15}N$ ± SD (‰)**	**Sample size (n)**	**Mean $\delta^{15}N$ ± SD (‰)**	**Sample size (n)**	**Mean $\delta^{15}N$ ± SD (‰)**	**Sample size (n)**	**Mean $\delta^{15}N$ ± SD (‰)**	**Sample size (n)**
Macrocystis pyrifera	8.99 ± 0.83	20	8.25 ± 2.22	23	8.50 ± 1.17	10	nd	nd	8.92 ± 0.57	25	5.80 ± 1.42	22
Nereocystis luetkeana	8.70 ± 1.15	20	6.58 ± 1.80	20	7.28 ± 1.57	14	nd	nd	8.28 ± 1.13	20	6.80 ± 1.75	27
Pterygophora californica	6.82 ± 1.04	20	nd	nd	6.84 ± 1.20	10	nd	nd	7.59 ± 0.79	15	nd	nd

Table 2.1. Regional summer and winter kelp mean $\delta^{13}C$ ± SD ‰ (section A.), $\delta^{15}N$ ± SD ‰ (section B.) and sample sizes for *Macrocystis*, *Nereocystis* and *Pterygophora*. During the winter, no data ("nd") was available for all three species in the otter-intermediate region, and for *Pterygophora* in the other regions.

2.3.5 Total POM (> 0.7 µm) carbon stable isotope values

Summer: The first principal component (PC1) accounted for 48.7 % of the total variance, and was characterized by a positive relationship among nitrate, phosphate and silica concentrations. The second principal component (PC2) accounted for 31.0 % of the total variance, and was characterized by the negative relationship between ocean depth, and total chl *a* concentration and SST. Contributing variables and their corresponding component loading are provided in Table 2.2. Total POM $\delta^{13}C$ values did not have a significant relationship with PC1 or PC2, however the relationship with PC1 was close ($R^2 = 0.11$, $p = 0.059$; Fig. 2.7 A), which was characterized by increasing total POM $\delta^{13}C$ values with decreasing nutrient concentrations. PC1 did not have a significant relationship with $\delta^{15}N$ and PC2 did not have a significant relationship with $\delta^{13}C$ or $\delta^{15}N$.

Winter: The winter total POM principal component analysis retained the first two components. PC1 accounted for 48.6 % of the total variance, while PC2 accounted for 22.2 %. PC1 was characterized by the positive relationship among total chl *a*, nitrate and phosphate concentrations, while PC2 was characterized by the negative relationship between silica concentration and SST (Table 2.2). PC1 had a significant relationship with total POM $\delta^{13}C$ ($R^2 = 0.18$, $p = 0.003$, Fig. 2.7 B), with $\delta^{13}C$ being more enriched with increased total chl *a*, phosphate and nitrate concentrations. These variables could potentially be linked through upwelling and vertical mixing during the winter months and providing surface waters with needed nutrients. Silica does not appear to be limiting in this system, and perhaps this is why it is not linked to the availability of the other two nutrients. PC1 did not have a significant relationship with $\delta^{15}N$, and PC2 did not have a significant relationship with $\delta^{13}C$ or $\delta^{15}N$.

2.3.6 20 – 63 µm POM carbon stable isotope values

Summer: PCA of summer 20 – 63 µm POM determined that PC1 accounted for 42.9 % of the total variance, while PC2 accounted for 35.7 %. PC1 was characterized by the positive relationship among total chl *a*, > 20 µm chl *a*, and sea surface temperature, while PC2 was characterized by the negative relationship between silica concentration, and phosphate concentration and ocean depth (Table 2.2). PC1 indicates that $\delta^{13}C$ increases as both chl *a* concentrations and SST increases. PC1 variables can potentially all be related spatially as phytoplankton concentration and SST decrease with distance from the kelp forest. When PC1 was plotted against $\delta^{13}C$ values there was a significant relationship ($R^2 = 0.23$, $p = 0.006$, Fig 2.7 C). PC1 did not have a significant relationship with $\delta^{15}N$ and PC2 did not have a significant relationship with $\delta^{13}C$ or $\delta^{15}N$.

Winter: PCA for winter 20 - 63 µm POM revealed that PC1 accounted for 48.6 % of the total variance, while PC2 accounted for 22.2 % (Table 2.2). PC1 consisted of a positive relationship among nitrate, phosphate, and > 20 µm chl *a* concentrations. PC1 increased with decreasing $\delta^{13}C$ values ($R^2 = 0.34$, $p = 0.002$; Fig. 2.7 D). This indicates that as $\delta^{13}C$ becomes more enriched with

the increasing concentration of these three variables. There was no relationship between neither PC1 and $\delta^{15}N$, nor PC2 and $\delta^{13}C$ or $\delta^{15}N$.

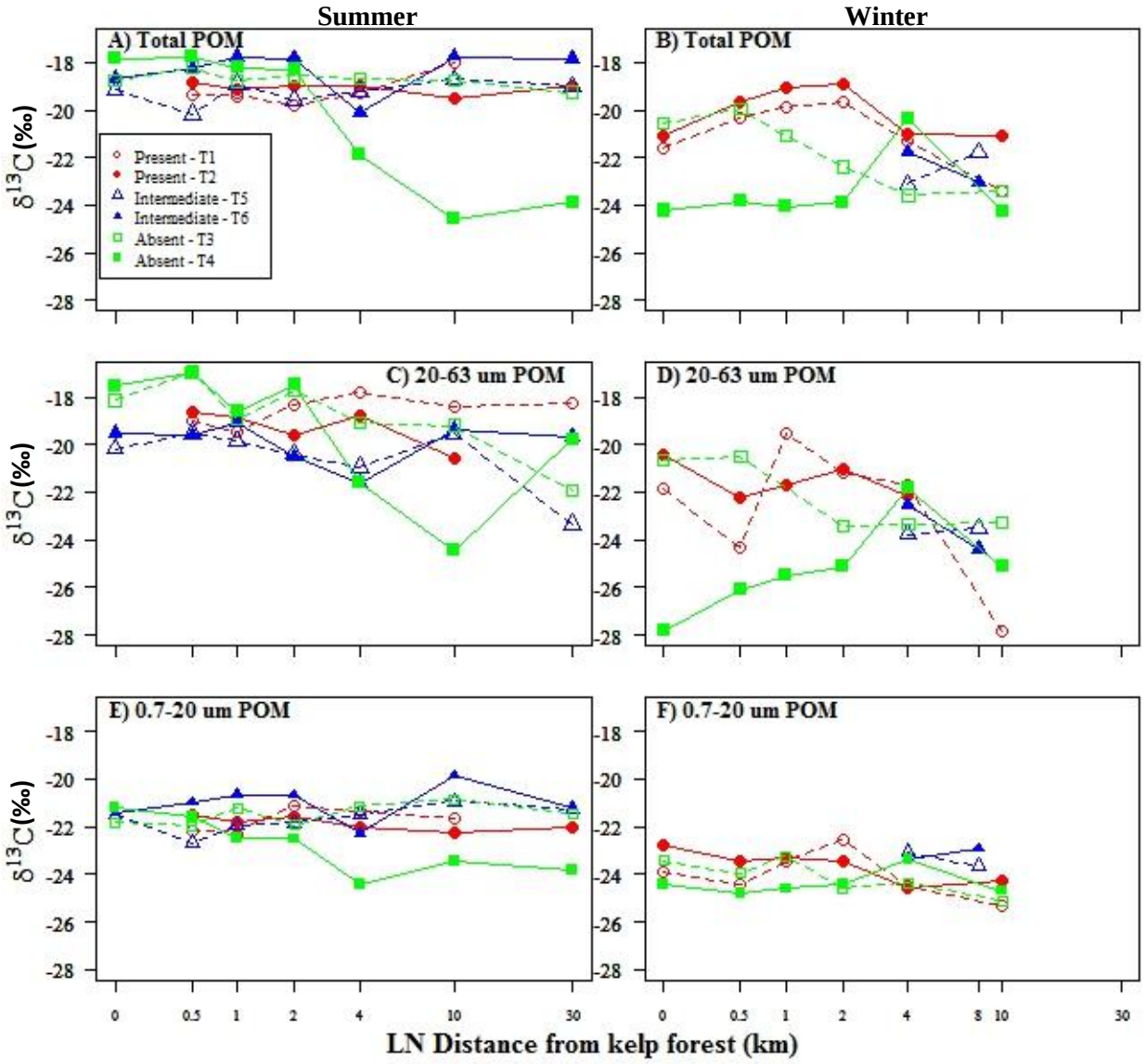

Figure 2.5. Summer (left column) and winter (right column) $\delta^{13}C$ values (‰) for total POM (>0.7 µm; panel A and B), 20 – 63 µm POM (panel C and D) and 0.7 – 20 µm POM (panel E and F) with natural log distance from the kelp forest for transects from all regions. Each point represents a single sample (n=1). For presentation purposes distance is in natural log scale.

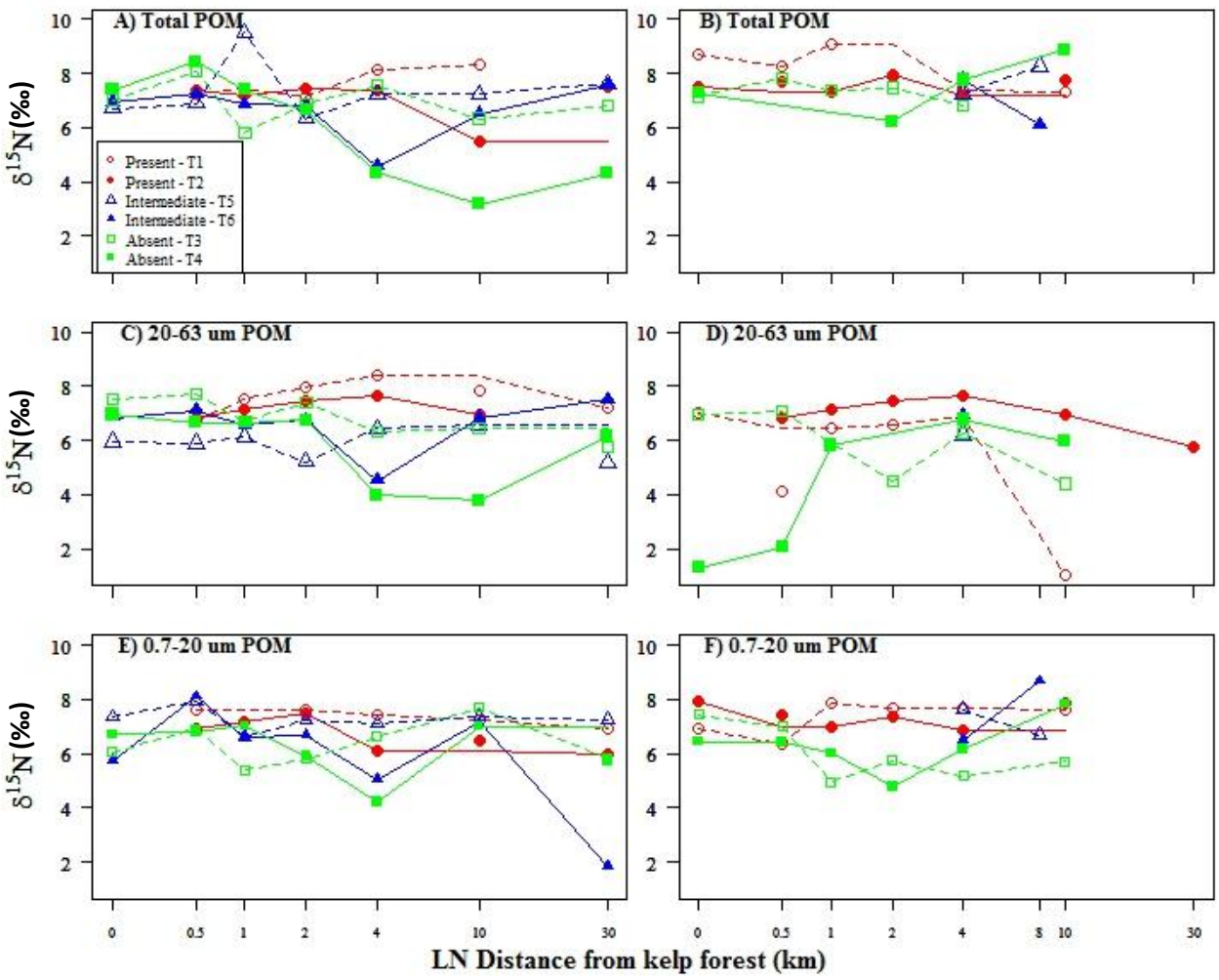

Figure 2.6. Summer (left column) and winter (right column) $\delta^{15}N$ values (‰) for total POM (>0.7 µm; panel A and B), 20 – 63 µm POM (panel C and D) and 0.7 – 20 µm POM (panel E and F) with natural log distance from the kelp forest for transects from all regions. Each point represents a single sample (n=1). For presentation purposes distance is in natural log scale.

Summer phytoplankton isotopes: To determine summer phytoplankton $\delta^{13}C$ values to use in mixing models for all size fractions I used chl *a* concentrations, nitrate concentrations, POM $\delta^{13}C$ and $\delta^{15}N$ values, and POM C:N ratios. The otter-absent region's offshore differences between transect 3 and 4 POM $\delta^{13}C$ values and chl *a* concentrations, and the associated relationships with the variables previously mentioned, allowed the estimation of appropriate size-fraction specific blooming and non-blooming phytoplankton values. From 4 to 30 km on transect 3 there were undetectable nitrate levels (Fig. 2.3 A). I assumed that this phytoplankton concentration was at or near maximum and characterized these concentrations as a "blooming phytoplankton". I used the $\delta^{13}C$ and $\delta^{15}N$ values at these stations for their respective size fractions for blooming phytoplankton in the mixing model (Table 2.4).

At the 10 and 30 km stations along transect 4, the phytoplankton concentration was relatively low (2.5 – 4.1 µg/L), but unfortunately nitrate concentration samples were not collected at these stations. However, depleted $\delta^{15}N$ values (3.2 – 5.2 ‰) indicate that this bloom could potentially be in the early stages. As Altabet and Francois (1994) pointed out, a phytoplankton bloom in its early stages will have depleted $\delta^{15}N$ values as it is utilizing $N^{14}O_3^-$ first and then utilizes $N^{15}O_3^-$ as nitrate becomes limiting. In contrast, at the selected distances for transect 3, $\delta^{15}N$ is enriched, indicative of increased incorporation of $N^{15}O_3^-$. For this reason, I characterized these values as "non-blooming phytoplankton" and used the corresponding $\delta^{13}C$ and $\delta^{15}N$ values for non-blooming phytoplankton in the mixing model (Table 2.4).

I was unable to determine regionally unique isotope values as the otter-present and otter-intermediate regions were experiencing high phytoplankton concentrations at most distances from shore and inhibited the contrast provided by the otter-absent transects. Additionally, the otter-present region has a regionally unique ocean current, which will be discussed later, that confounded the selection of appropriate isotope values. Therefore, I used the blooming and non-blooming phytoplankton values described above for all analyses.

Winter phytoplankton isotopes: Along transect 4 in the otter-absent region the 0 to 1 km station 20 – 63 µm POM isotope values were highly depleted ($\delta^{13}C$, -25.5 to -27.8 ‰; $\delta^{15}N$, 1.3 to 2.1 ‰). CTD salinity data indicated that surface water was relatively quite fresh with salinity measurements between 26 and 29 ppt (data not present here). This likely means that there was a terrestrial influence in this region therefore these values were not included. For consistency, I did not include the total POM and 0.7 – 20 µm POM values at these same stations even though these fractions did not seem to be affected by the terrestrial input, i.e. relatively more depleted. For transect 3, I did not include 0 to 1 km station data for all POM isotope values to avoid possible kelp detritus influence in the resulting values. Therefore, I determined $\delta^{13}C$ and $\delta^{15}N$ means along both transects that were further offshore than 1 km (Table 2.4). Certain $\delta^{15}N$ was not used in these calculations as there was not enough nitrogen present in the POM sample to retrieve a reliable value. As with summer calculations, data from the otter-present region was not used for the reasoning provided above.

Oceanic phytoplankton stable isotope $\delta^{13}C$ and $\delta^{15}N$ values were obtained from surface seawater total POM sampling that took place onboard the CCGS *John P. Tully* in September 2009 up to approximately 120 km off the WCVI. Resulting mean $\delta^{13}C$ and $\delta^{15}N$ isotope values were -26.11 ± 0.36 ‰ (n = 3) and 2.88 ± 0.40 ‰ (n = 2), respectively.

The isotope values selected for 0.7 – 20 µm phytoplankton were not used in the 20 – 63 µm POM analyses because I assumed this plankton size was not retained by the 20 µm filter. Blooming phytoplankton isotope values were not considered for winter POM analyses as phytoplankton blooms were not occurring.

	Summer total POM		Summer 20 – 63 µm POM		Winter total POM		Winter 20 – 63 µm POM	
Significant relationships	$\delta^{13}C$: $R^2 = 0.11$ $p = 0.059$	None	$\delta^{13}C$: $R^2 = 0.23$ $p = 0.006$	None	$\delta^{13}C$: $R^2 = 0.18$ $p = 0.003$	None	$\delta^{13}C$: $R^2 = 0.34$ $p = 0.002$	None
	PC1	**PC2**	**PC1**	**PC2**	**PC1**	**PC2**	**PC1**	**PC2**
Cumulative % Variance	48.7	79.7	42.9	78.6	48.6	70.8	46.0	76.1
$[NO_3^- + NO_2^-]$	-0.56	-0.04	-	-	0.60	-0.16	-0.60	0.12
$[PO_4^-]$	-0.56	-0.05	-0.22	-0.50	0.57	-0.22	-0.60	0.07
$[SiO_2]$	-0.49	-0.24	0.04	0.61	0.26	0.56	-	-
Ocean depth	0.17	0.56	0.06	-0.60	-	-	0.05	0.71
SST	0.24	-0.60	-0.52	0.10	-0.14	-0.78	0.16	0.69
Total [chl *a*]	0.22	-0.51	-0.60	0.02	0.47	-0.09	-	-
20 µm [chl *a*]	-	-	-0.56	0.07	-	-	-0.50	0.05

Table 2.2. Summer and winter total (>0.7 µm) and 20 – 63 µm POM PCA component loadings for PC1 and PC2 and their cumulative variance (%). Variables that did not load (>|0.4|) on PC1 or PC2 are indicated by "-".

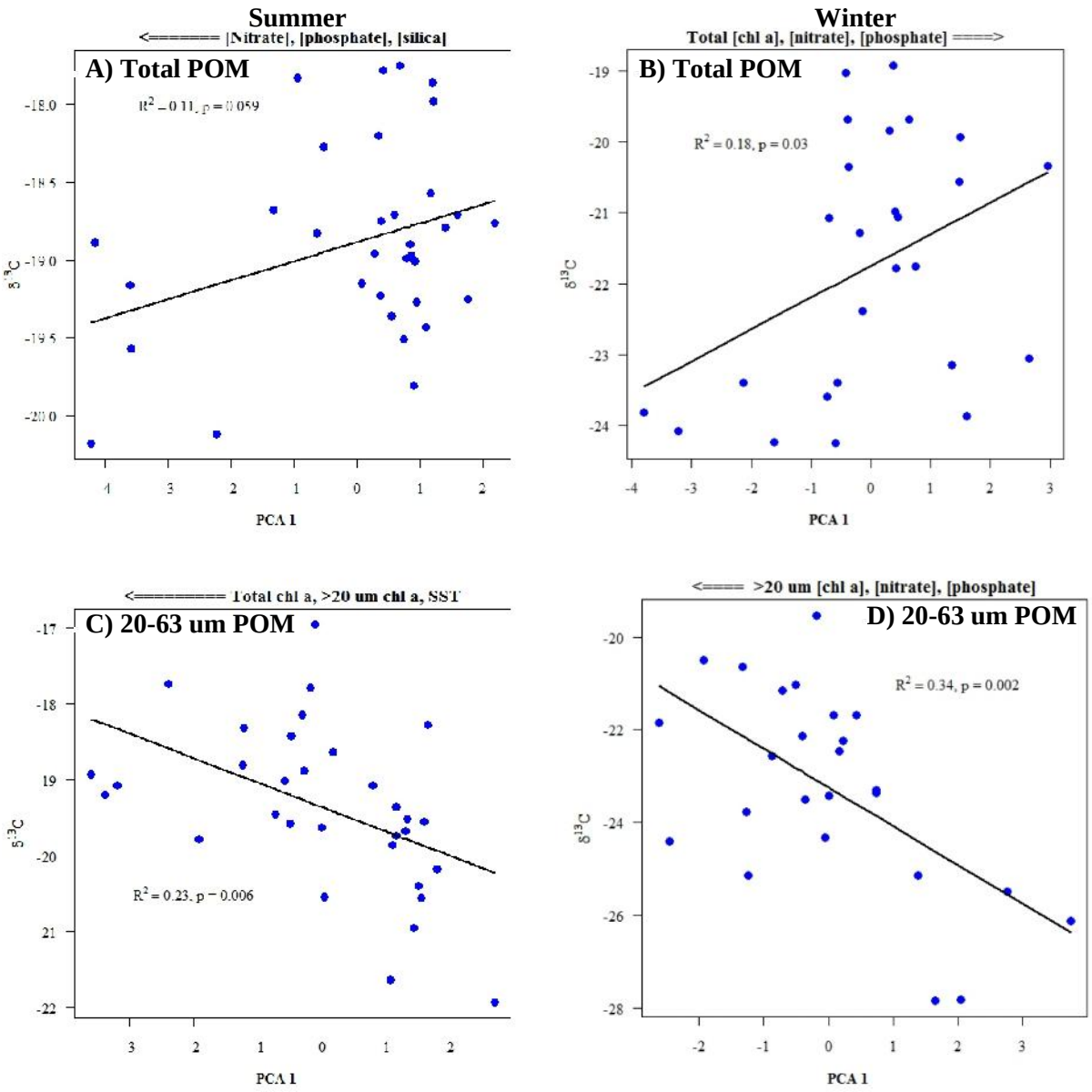

Figure 2.7. Relationships between PC1 values and summer and winter POM $\delta^{13}C$ values. Arrows indicate direction along the component axis with which there is a correlation. Summer total and 20 – 63 µm POM (panels A and C, respectively) and winter total and 20 – 63 µm POM (panels B and D, respectively). Arrows indicate direction of positive increase for associated variables.

	Otter-present ***Macrocystis* – 66.7%,** ***Nereocystis* – 33.3%**		**Otter-intermediate** ***Nereocystis* – 80%,** ***Pterygophora* – 20%**		**Otter-absent** ***Nereocystis* – 100%**	
Season	**Mean $\delta^{13}C$ ± SD (‰)**	**Mean $\delta^{15}N$ ± SD (‰)**	**Mean $\delta^{13}C$ ± SD (‰)**	**Mean $\delta^{15}N$ ± SD (‰)**	**Mean $\delta^{13}C$ ± SD (‰)**	**Mean $\delta^{15}N$ ± SD (‰)**
Summer	-14.55 ± 1.37	8.87 ± 0.93	-16.68 ± 1.70	7.19 ± 1.50	-18.31 ± 1.17	8.28 ± 1.13
Winter	-15.68 ± 2.29	7.69 ± 2.08	-15.58 ± 2.30	5.77 ± 1.66	-16.15 ± 2.27	6.80 ± 1.75

Table 2.3. Summer and winter kelp $\delta^{13}C$ and $\delta^{15}N$ values (‰) ± SD used in MixSIR based on the regionally abundant kelp species' proportions (Martone and Markel, unpublished data). Winter *Pterygophora* data are calculated based on the average $\delta^{13}C$ and $\delta^{15}N$ depletion from summer to winter experienced by *Macrocystis* and *Nereocystis* and then applied to the otter-intermediate region.

		Summer		Winter	
POM size fraction	**Phytoplankton type**	**Mean $\delta^{13}C$ ± SD (‰)**	**Mean $\delta^{15}N$ ± SD (‰)**	**Mean $\delta^{13}C$ ± SD (‰)**	**Mean $\delta^{15}N$ ± SD (‰)**
Total POM	Blooming phytoplankton >0.7 µm	-18.91 ± 0.30	6.9 ± 0.63	No bloom	No bloom
	Non-blooming phytoplankton >0.7 µm	-24.23 ± 0.48	3.74 ± 0.80	-23.5 ± 0.70	7.81 ± 1.03
	Blooming 0.7 – 20 µm phytoplantkon	-21.15 ± 0.31	6.71 ± 0.93	No bloom	No bloom
	Non-blooming 0.7 – 20 µm phytoplantkon	-23.63 ± 0.28	6.37 ± 0.91	-24.42 ± 0.58	5.9 ± 1.06
	Oceanic phytoplankton >0.7 µm	-26.11 ± 0.36	2.88 ± 0.40	-26.11 ± 0.36	2.88 ± 0.40
20 – 63 µm POM	Blooming phytoplankton 20 – 63 µm	-19.36 ± 0.38	6.31 ± 0.15	No bloom	No bloom
	Non-blooming phytoplankton 20 – 63 µm	-23.9 ± 0.78	4.47 ± 0.96	-23.7 ± 1.26	5.85 ± 1.19
0.7 – 20 µm POM	Blooming phytoplankton 0.7 – 20 µm	-21.15 ± 0.31	6.71 ± 0.93	No bloom	No bloom
	Non-blooming phytoplankton 0.7 – 20 µm	-23.63 ± 0.28	6.37 ± 0.91	-24.42 ± 0.58	5.9 ± 1.06

Table 2.4. Summer and winter phytoplankton $\delta^{13}C$ and $\delta^{15}N$ values (‰ ± SD) used in the MixSIR isotope mixing model program for the associated size fraction of POM.

2.3.7 Spatial and temporal distribution of kelp-derived detritus

Summer: During the summer in the otter-present region, median KDD contributions to total POM were between 30 % and 55 % (9 to 71 % possible contribution range; Fig 2.8, panel A). The median contributions to 20 – 63 POM were from 11 to 63 % (1 to 82 % possible contribution range; Fig. 2.9, panel A). Median KDD contribution to total POM was greatest

along transect 1 for all size fractions with a maximum at 10 km from shore for total POM and at 4 km from shore for 20 – 63 µm and 0.7 – 20 µm POM. In general, the median percent contribution to total and 20 – 63 µm POM remained moderate to high and constant at all distances from shore. The median contribution to 0.7 – 20 µm POM remained low and constant at all distances from the kelp forest. Medians were from 7 to 17 % with a possible range of 1 to 31 % (Fig. 2.10, panel A), which was much lower than the two larger size fractions, in general. Within a given size fraction there was a high degree of confidence interval overlap within and between transects indicating that there was a high probability that KDD contributions were similar with distance from the kelp forest and between transects.

There was moderate overlap in confidence intervals within the otter-intermediate region for all POM size fractions again indicating that there was low probability of KDD contribution differences with distance and between transects. However, there was greater variability with distance and between transects compared to the otter-present region. The median KDD contributions to total POM were from 26 to 66 % (1 to 82 %, possible range; Fig. 2.8), while median contributions to 20 – 63 µm POM were from 15 to 54 % (1 to 80 %, possible range; Fig. 2.9, panel A). The 20 – 63 µm POM confidence intervals consistently ranged from low to high KDD contribution to POM across most distances from the kelp forests except for the 30 km on transect 5 where the confidence interval ranged from 33 to 80 %. In general, this region has the most uncertainty (i.e. large confidence intervals) in the contribution of KDD relative to the other fractions regions which is at least partly due to the standard deviations of the kelp contributors being larger (Table 2.3). The median KDD contribution to 0.7 – 20 µm POM was low (8 %) to moderate (40 %) at all distances except along transect 6 at 0.5 and 10 km from shore, where the maximum possible contribution was as high as 59 and 67 %, respectively (Fig. 2.10, panel A).

During the summer along the otter-absent region transects, median KDD contribution varied greatly among size fractions and between transects. Total and 20 – 63 µm POM within this region had the least confidence interval overlap which indicates there was a higher probability of differences in contribution with distance from the kelp forest and between transects at a given distance (Fig. 2.8 and 2.9, panel A). The median KDD contributions to total POM along transect 4 decreased dramatically with distance from the kelp forest. This was also the case for 20 – 63 µm POM but also occurred along transect 3. However, further offshore there was greater uncertainty along transect 3. Total POM Median KDD contributions were greatest in this region, relative to the two other regions, with the highest being 71 % (56 to 85 %, possible contribution) for total POM and 82 % (62 to 95 %, possible contribution) for 20 – 63 µm POM. For the same two fractions (total and 20 – 63 µm POM), this region had the lowest median KDD contributions of 3 % (0 to 9 % C.I.) and 3 % (0 to 12 % C.I.), respectively. The median contribution to the 0.7 – 20 µm POM remained low and constant with distance from the kelp forest with the exception at 10 km along transect 3. For this fraction the median contributions were from 1 to 37 % (0 to 63 %, possible range; Fig. 2.10, panel A). Similar to the other regions,

median KDD contributions were largest for total and 20 – 63 µm POM relative to the smallest size fraction (0.7 – 20 µm POM).

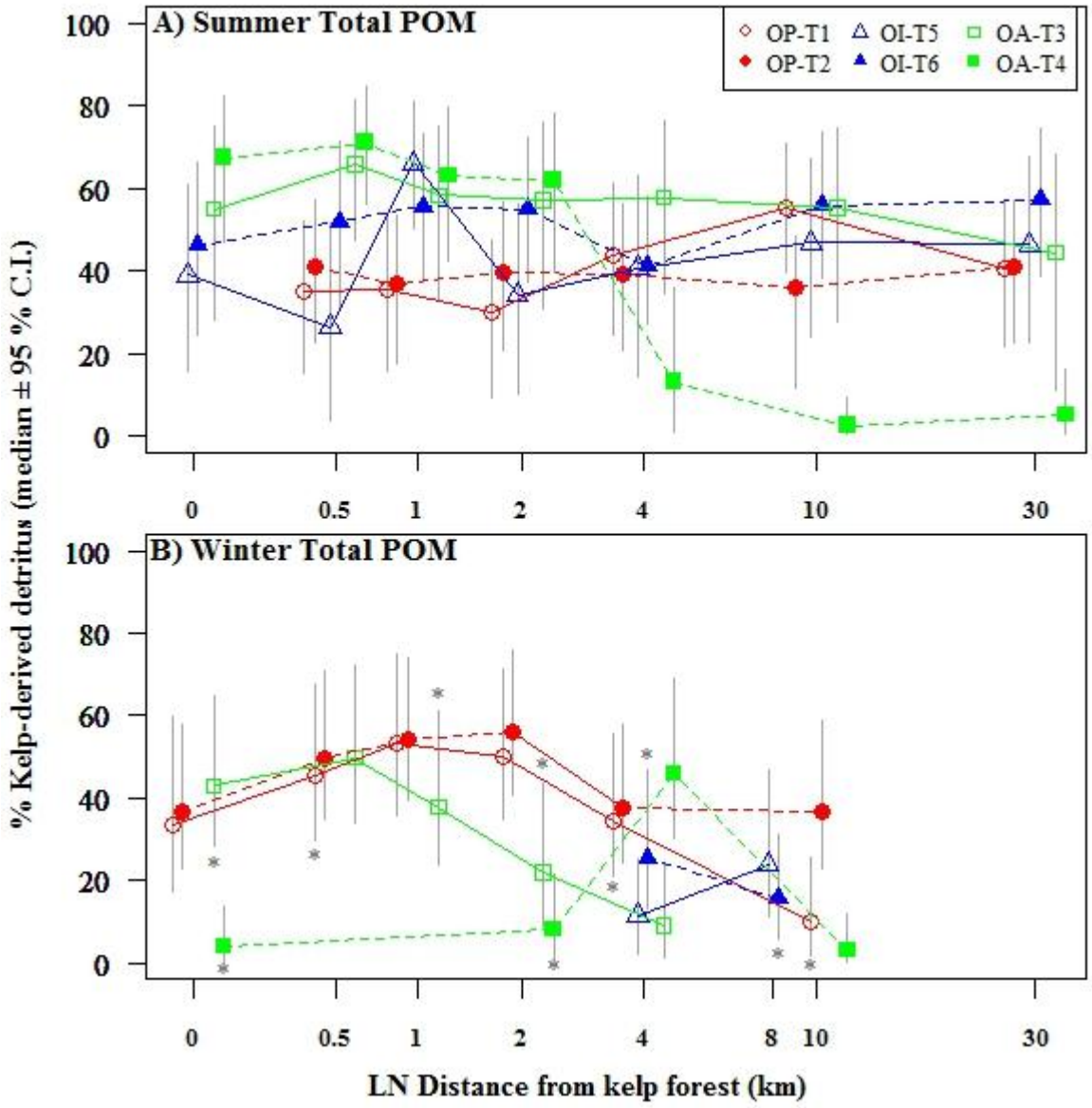

Figure 2.8. Summer (panel A) and winter (panel B) percent kelp-derived detritus contribution (median ± 95 % C.I.) to total POM (>0.7 µm) determined by MixSIR. OP = otter-present region, OI = otter-intermediate region, and OA = otter-absent region. Each region is followed by their respective transect numbers. The "*" denote $\delta^{15}N$ data that was determined to be "at the limits of linearity". For display purposes points are jittered horizontally and distance is in natural log scale.

Winter: During the winter in the otter-absent region, median KDD contributions to total POM were from 10 to 56 % (2 to 76 %, possible range) and there was generally large overlap in confidence intervals at most distances from the kelp forest indicating that there was low probability that the contribution changed with distance (Fig. 2.8, panel A). Median KDD contribution was at its highest at 2 km along transect 2 at 56 % (41 to 76 % C.I.), which was the highest among all size fractions within this region. The KDD contribution medians for 20 – 63 µm POM were from 1 to 53 % (1 to 85 %, maximum possible contribution range). This fraction had the most variability with distance with respect to median KDD contributions and had relatively large confidence intervals (Fig. 2.9, panel B). The 0.7 – 20 µm POM had a maximum contribution at 2 km from the kelp forest with median of 23 % (10 to 48 % C.I.; Fig. 2.10, panel B). In general, median KDD contributions to this fraction remained below 20 % and constant.

Only 2 distances along each transect were sampled in the otter-intermediate region during the winter (Figs. 2.8 to 2.10). The median KDD contribution at 4 and 8 km from the kelp forest was similar between transects for all size fractions. The median contributions to total POM were from 11 to 25 % (2 to 47 %, maximum possible range). The largest median KDD contribution to 20 – 63 µm POM was 53 % and a minimum of 1 % (1 to 86 %, maximum possible range; Fig. 2.9, panel B). Median contributions to 0.7 – 20 µm POM were 10 to 15 % (2 to 33 %, maximum possible range; Fig. 2.10, panel B).

In the otter-absent region there was a lot of variation between transects and with distance from the kelp forest. The median percent KDD contributions to transect 3 were always larger within 2 km from the kelp forest for total and 20 – 63 µm POM. Median KDD contributions along transect 3 from 0 to 2 km were from 22 to 50 % (9 to 72 %, maximum possible contribution range; Fig. 28, panel B). In contrast, median KDD contributions to transect 4, within this same range of distances, were 4 to 8 % (1 to 21, maximum possible range). In general, this difference also existed for the 20 – 63 µm POM (Fig. 2.9, panel B). With the exception of transect 4 increasing to a peak at 4 km from the kelp forest, Median KDD contributions along transect 3 decreased with distance from the kelp forest, while the transect 4 median peaked at 4 km and then decreased again at 10 km for all size fractions. Median KDD contributions to 0.7 – 20 µm POM were low and constant with distance from the kelp forest. Within this fraction medians were from 2 to 14 % (0 to 34 %, maximum possible range; Fig. 2.10, panel B).

Summer-Winter Comparison: In general during the winter, median KDD contributions were lower by as much as 68 %, in the case of total POM, when compared to summer contributions and there was much more variability in median contributions with distance from the kelp forest. During the winter were also smaller confidence intervals compared to the summer, which was the case for all regions and size fractions.

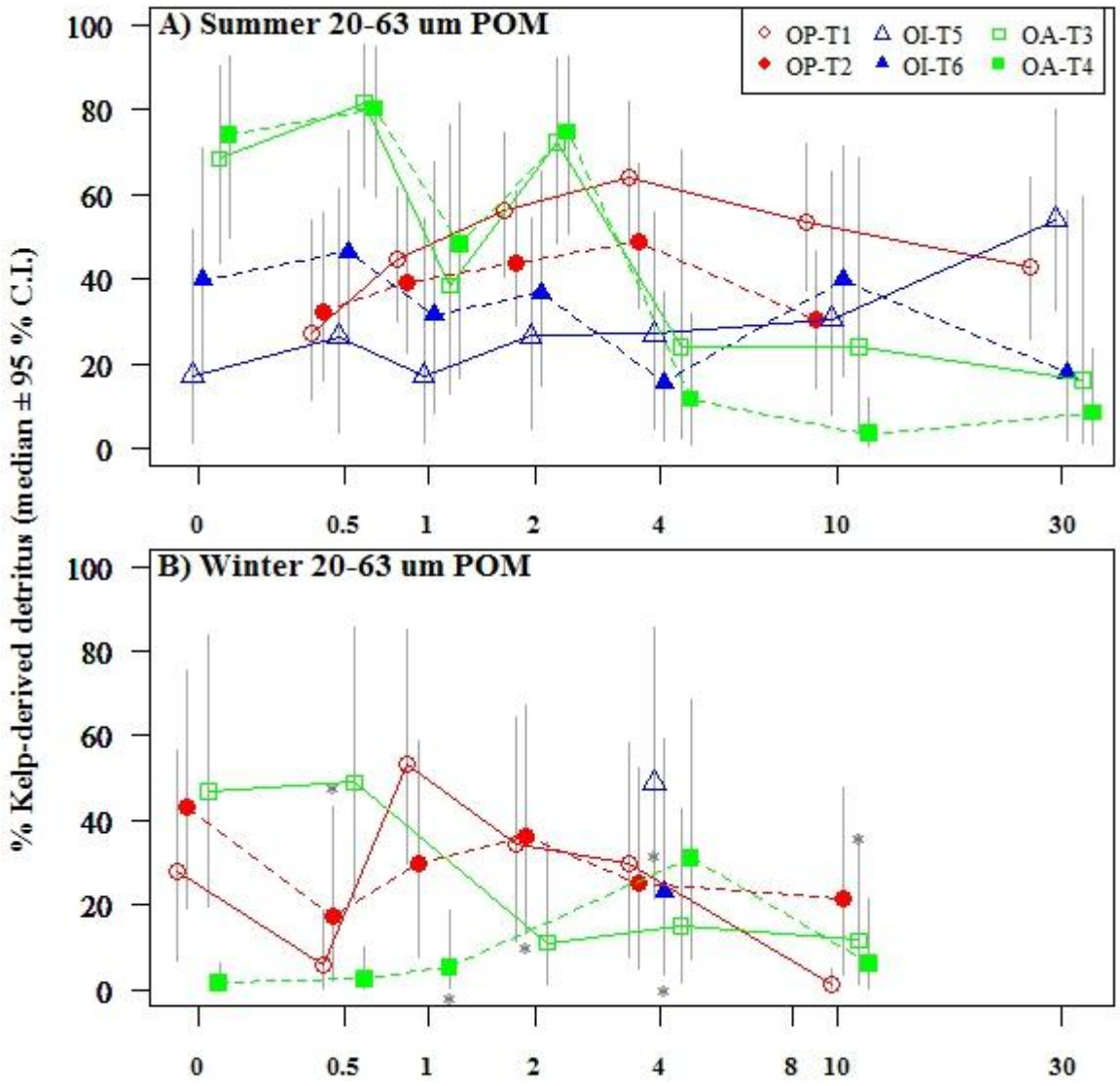

Figure 2.9. Summer (panel A) and winter (panel B) percent kelp-derived detritus contribution (median ± 95 % C.I.)to 20 – 63 µm POM determined by MixSIR. OP = otter-present region, OI = otter-intermediate region, and OA = otter-absent region. Each region is followed by their respective transect numbers. The "*" denote $\delta^{15}N$ data that was determined to be "at the limits of linearity". For display purposes points are jittered horizontally and distance is in natural log scale.

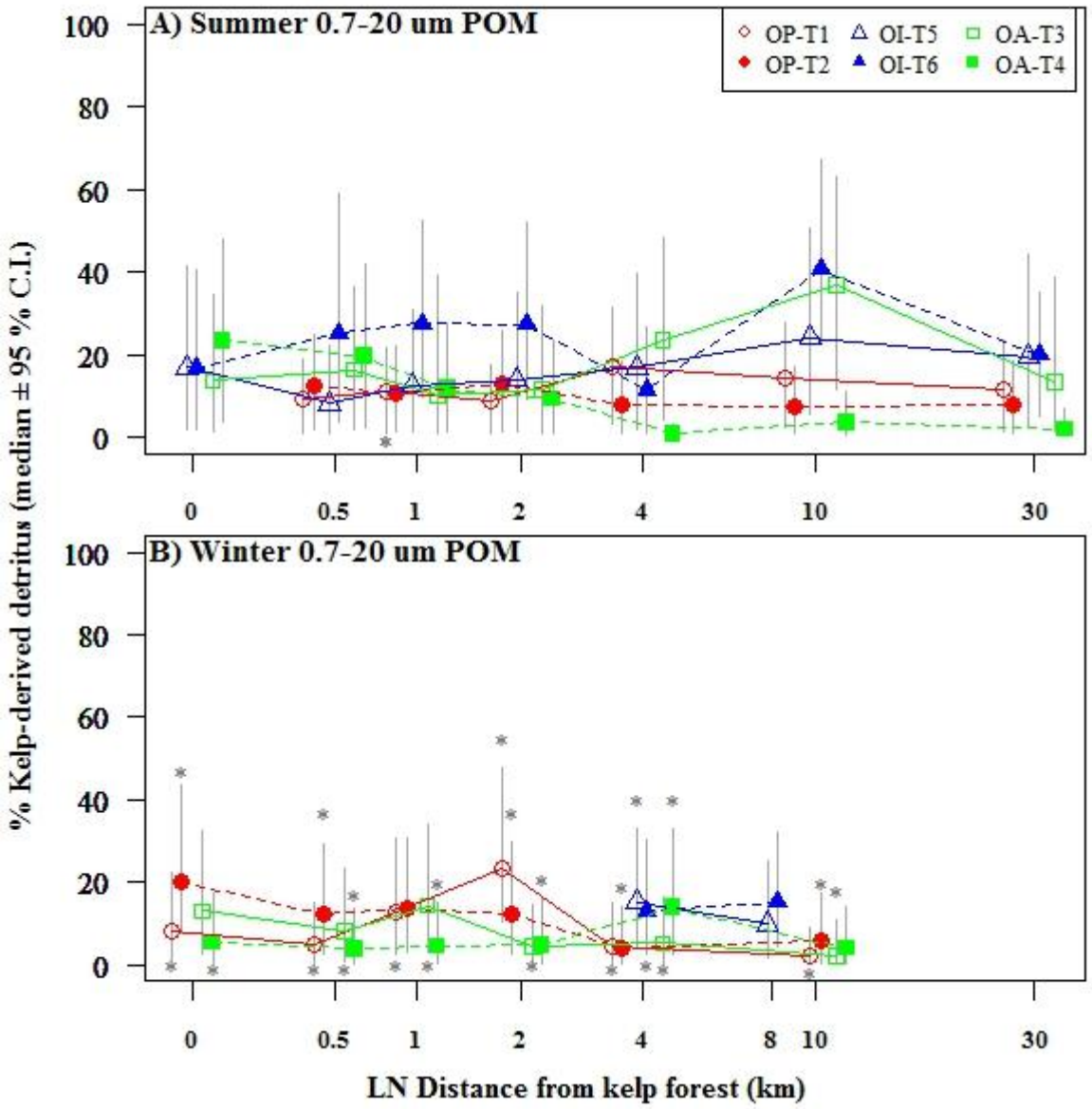

Figure 2.10. Summer (panel A) and winter (panel B) percent kelp-derived detritus contribution (median ± 95 % C.I.) to 0.7 – 20 µm POM ± 95 % CI determined by MixSIR. OP = otter-present region, OI = otter-intermediate region, and OA = otter-absent region. Each region is followed by their respective transect numbers. The "*" denote $\delta^{15}N$ data that was determined to be "at the limits of linearity". For display purposes points are jittered horizontally and distance is in natural log scale.

2.4 Discussion

Overall, we found no differences in KDD contribution to summer POM among regions differing in kelp abundance; however, we detected a difference between transects in the otter-absent region for total and 20 – 63 µm POM offshore. During the winter in the otter-present region, the possible contribution of KDD remained high relative to summer months. This was in contrast to the otter absent region where the possible contribution of KDD decreased in winter months. In general during summer and winter, total and 20 – 63 µm POM had a larger KDD contribution compared to 0.7 – 20 µm POM.

2.4.1 Kelp isotopic variability across species, seasons and regions

Within region differences in kelp isotope values were prevalent in this study which could have implications for estimated KDD contribution (Table 2.1, Fig. 2.4). During the summer, the otter-present and absent regions' *Macrocystis* had enriched $\delta^{13}C$ values compared to *Nereocystis* and *Pterygophora* (Fig. 2.4). For $\delta^{15}N$, the otter-present region *Macrocystis* and *Nereocystis* were more enriched than *Pterygophora* while in the otter-absent region *Macrocystis* was more enriched than *Nereocystis* and *Pterygophora*, and *Nereocystis* was more enriched than *Pterygophora*. During the winter, the otter-absent region *Macrocystis* $\delta^{13}C$ was enriched compared to *Nereocystis*. In the otter-present region *Macrocystis* $\delta^{15}N$ was more enriched than *Nereocystis*. All three species of kelp within a region were sampled within 1 to 3 days so large physiological changes related to seasonal and abiotic factors should have been negligible (Simenstad et al. 1993). Time of kelp recruitment, growth chronology, and age composition of the kelp forests sampled (*Macrocystis* and *Pterygophora* are perennials, and *Nereocystis* is an annual) may be region or site-specific. These factors affect differences in metabolism and storage of biochemical compounds with differing $\delta^{13}C$ values on shorter time scales (Stephenson et al. 1984, Simenstad et al. 1993). In this study, all samples were systematically collected from distal ends of the blade from adult plants at the ocean surface. If species distribute $\delta^{13}C$ differently among the blade tissue then the sampling procedure could lead to biases. With respect to $\delta^{15}N$ values, relatively low nitrate concentrations within and nearby specific kelp forests sites prior to sampling may explain within and among region differences. When nitrate is abundant, kelps preferentially incorporate ^{14}N into their tissues (Ostrom et al. 1997). As localized nutrients are consumed within kelp forests due to higher kelp and phytoplankton photosynthetic rates, especially during the summer, thus decreasing ^{15}N discrimination leading to an increase in the kelp $\delta^{15}N$, reflecting the source $\delta^{15}N$ (Altabet and Francois 1994). This spatial variation in nitrate concentration, source, and utilization could lead to the differences described here.

In the present study, the $\delta^{13}C$ and $\delta^{15}N$ values of summer *Macrocystis* from the otter-present region were more enriched than during the winter. In contrast, summer *Nereocystis* from the otter-absent region had depleted $\delta^{13}C$ relative to winter samples; however, *Nereocystis* $\delta^{15}N$ values were enriched relative to winter samples in both the otter absent and otter-absent regions

(Table 2.1, Fig. 2.4). In California, $\delta^{13}C$ and $\delta^{15}N$ values of *Macrocystis* have been shown to be more depleted from March to July compared to samples collected from August to December (Foley and Koch 2010), which is the opposite of what was found in this study. The summer-winter *Macrocystis* $\delta^{13}C$ difference could be due to highly favourable conditions for growth (i.e. increased solar radiation) during the summer and increased photosynthetic rates as kelp are capable of depleting the available CO_2 at the blade's surface leading to enriched $\delta^{13}C$ values (Farquhar et al. 1989). However, it is unclear why this was only the case in the otter-present region. Dissolved inorganic carbon ($CO_{2(aq)}$ or HCO_3^-) $\delta^{13}C$ has been shown to vary spatially and temporally in estuaries (Fry and Sherr 1984).The potential differential utilization of these carbon sources (Lucas and Berry 1985, Prins and Elzenga 1989) of differing $\delta^{13}C$ values (Mook et al. 1974) could lead to the seasonal differences shown here. Foley and Koch (2010) found significantly lower $\delta^{13}C$ values in *Macrocystis* when nitrate concentrations were high, which could be the case in this study with high nitrate concentrations within and nearby the kelp forests during the winter depleting $\delta^{13}C$ values. Variability in the isotopic composition of biochemical molecules such as fatty acids and lipids has been used as an explanation for $\delta^{13}C$ variation between seasons (Stephenson et al. 1984, Dunton and Schell 1987). Additionally, longer time scale alongshore differences in sea surface temperature (Harris 2001) among regions as a result enhanced influence of upwelled water due to differences in continental shelf width along the coast (Thomson et al. 1989) could be supplying the otter-present region with colder, and therefore more ^{13}C enriched CO_2. Relatively low nitrate concentrations within and nearby the kelp forests during the summer may explain why summer kelp samples have relatively enriched $\delta^{15}N$ values. Localized nutrient drawdown within kelp forests during the summer due to higher kelp and phytoplankton photosynthetic rates results in the decreased discrimination of $\delta^{15}N$ leading to an increase in the kelp $\delta^{15}N$ (Altabet and Francois 1994). Furthermore, it has been shown that nitrogen content of kelp varies during the growing cycle (Sjøtun et al. 1996) and, as mentioned previously, isotope ratio differences in parts of kelp lamina or at different sampling times could be caused by different biochemical reactions during the growth cycle (Fredriksen 2003).

The only among region isotope value differences of a similar species were the summer $\delta^{15}N$ of *Nereocystis* between the otter-present and intermediate regions, and the winter *Macrocystis* $\delta^{15}N$ between otter-present and absent regions. This indicates that, for the most part, abiotic conditions were similar among regions at the time of sampling even though the different regions were sampled during different months within each season. Furthermore, isotope values are integrated over longer time scales and the abiotic measurements (e.g. nitrate concentrations) taken in this study are a snapshot in time (Stephenson et al. 1984). It is unclear why these differences existed; however, remineralized nitrate has depleted $\delta^{15}N$ relative to upwelled nitrate, perhaps indicating that there was a regional difference in the available nitrate source (Fig. 2.3; Altabet 1988). Moreover, the degree of fractionation will be lower with more rapid growth and lower nitrogen concentrations perhaps indicating that there were regional differences with

respect to these two factors as the kelp samples were not all from within the vicinity of other measurements (Wada and Hattori 1978, York et al. 2007).

The collection of kelp from the distinct regions and different seasons revealed the spatial and temporal variability that can exist in both stable isotope values. These findings highlight the need to determine spatially and temporally unique kelp isotope values and the relative proportions locally abundant kelp species contribute to POM. Kelp summer $\delta^{13}C$ and $\delta^{15}N$ mean values from this study ranged from -13.2 to -19.5 ‰ and 6.8 to 9.0 ‰, respectively, whereas winter kelp $\delta^{13}C$ and $\delta^{15}N$ mean values ranged between -15.4 to -16.3 ‰ and 5.8 to 8.3 ‰. These ranges fall within other isotope studies of kelp in the northeast Pacific Ocean where $\delta^{13}C$ range from -12 to -20.5 ‰ (Page et al. 2008, Foley and Koch 2010). Similarly, $\delta^{15}N$ mean values are known to range between 5 and 9 ‰ (Foley and Koch 2010).

2.4.2 Phytoplankton isotopic variability between seasons and distance offshore

Determining unique isotope values for the different size fractions of blooming and non-blooming phytoplankton was critical in obtaining the most accurate estimates of KDD to POM. PCA results for winter total POM, and summer and winter 20 – 63 μm POM indicate that at least one chl *a* concentration size fraction explains each respective size fraction's POM $\delta^{13}C$ variability (Table 2.2, Fig. 2.7). During summer, the offshore differences in the otter-absent region between transect 3 and 4 POM $\delta^{13}C$ values and chl *a* concentrations allowed for the determination of appropriate summer phytoplankton $\delta^{13}C$ values for all size fractions. Undetectable nitrate levels along transect 3 from 4 to 30 km and relatively very high chl *a* concentrations (11.7 – 15.1 μg/L; Fig. 2.2, panel A, and 2.3 panel A) led to the conclusion that this phytoplankton concentration was at, or near, maximum and defined it as a "phytoplankton bloom". Therefore, I used the $\delta^{13}C$ and $\delta^{15}N$ values at these stations for their respective size fractions for blooming phytoplankton in the mixing model. Phytoplankton concentrations were much lower (2.5 – 4.1 μg/L) along transect 4 at the 10 and 30 km stations. The $\delta^{15}N$ values are quite depleted (3.2 – 5.2 ‰) at these stations along transect 4 which indicates that this bloom was likely in its early stages (Fig. 2.6, panel A). Phytoplankton blooms in their early stages have depleted $\delta^{15}N$ values because phytoplankton utilize $N^{14}O_3^-$ first and then utilizes $N^{15}O_3^-$ as nitrate becomes limiting (Altabet and Francois 1994). In contrast, at the selected distances for transect 3 $\delta^{15}N$ is enriched, indicative of increased incorporation of $N^{15}O_3^-$ and within reported upwelled nitrate $\delta^{15}N$ values for the northeast Pacific Ocean (Miyake and Wada 1967). Consequently, I defined it as "non-blooming phytoplankton" and used corresponding $\delta^{13}C$ and $\delta^{15}N$ values for non-blooming phytoplankton in the mixing model. C:N ratios on both transects at these distances were not different and were relatively low (5.4 – 6.2; Appendix A1), presumably because these phytoplankton were growing fast under nutrient replete conditions (Goldman et al. 1979, Kiørboe 1989). However, with the assumption that at 10 and 30 km along transect 4 were still in the early stages of blooming (Fig. 2.2, panel A), these ratios are possible. Furthermore, kelp

detritus influence is likely very low at all offshore distances mentioned as C:N ratios were generally < 6, and kelp sampled in this study had mean ratios > 10.5 (Appendix A2).

The various size fractions of phytoplankton collected in this study had different $\delta^{13}C$ and $\delta^{15}N$ values but there was little difference between seasons within a size fraction. Blooming phytoplankton was always more enriched than its associated non-blooming size fraction, and blooming size fractions that incorporated larger phytoplankton always had the most enriched $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 2.5 and 2.6). Blooming summer phytoplankton > 0.7 µm, that was comprised mostly of phytoplankton > 20 µm, had the most enriched $\delta^{13}C$ (-18.91 ± 0.3 ‰) and $\delta^{15}N$ (6.9 ± 0.63 ‰) values. This fraction's non-blooming counterpart had $\delta^{13}C$ that was approximately 5 ‰ more depleted (-24.23 ± 0.48 ‰) and had a $\delta^{15}N$ value that was greater than 3 ‰ more depleted (3.74 ± 0.80 ‰). There was a similar pattern and values for the 20 – 63 µm phytoplankton. The 0.7 – 20 µm blooming phytoplankton was the most depleted relative to the other fractions (-21.15 ± 0.31 ‰ and 6.71 ± 0.93 ‰) and had a similar non-blooming $\delta^{13}C$ value (-23.63 ± 0.28 ‰) as the other fractions but was more than 2 ‰ more enriched (6.37 ± 0.91 ‰) in ^{15}N compared to the other fractions. These values are similar to other studies of phytoplankton $\delta^{13}C$ (Duggins et al. 1989, Fry and Wainright 1991, Perry et al. 1999).

Phytoplankton $\delta^{13}C$ values can vary as a result of cell growth rate (Rau et al. 1992), ambient $CO_{2(aq)}$ concentration (Rau et al. 1991), plankton species composition (Fry and Wainright 1991), the type of carboxylation enzyme utilized during photosynthesis (RuBisCO versus PEPCase; Fontugne et al. 1991), irradiance, day length (Thompson and Calvert 1994), active inorganic carbon uptake (Raven et al. 1993), and sea temperature (Wong and Sackett 1978). Surface POM $\delta^{13}C$ was correlated to higher chl *a* concentrations off the coast of California and Washington, which was attributed to a greater abundance of diatoms nearshore of an upwelling front where chl *a* measures were >5 µg/L with smaller phytoplankton (<5 µm coccooid cyanobacteria and eukaryotic phytoplankton) being offshore of the upwelling front, where chl *a* concentrations were lower (Sherr et al. 2005, Miller et al. 2008). Similarly, blooming phytoplankton (e.g. Fry and Wainright 1991) and higher sea surface temperatures (e.g. Francois et al. 1993) are correlated to enriched $\delta^{13}C$ values. In this study, where the dominant phytoplankton group was assumed to be diatoms due to the time of year and findings from previous studies (Mackas and Sefton 1982, Harris 2001), PCA showed that chl *a* concentration was positively correlated with POM $\delta^{13}C$ which supports these previous findings. Perhaps, due to ^{13}C enriched upwelled summer water, phytoplankton $\delta^{13}C$ would be more enriched during the summer relative to winter, but this was not the case (Rau et al. 1989, 1991, 1992).

The $\delta^{15}N$ values for the various phytoplankton size fractions determined in this study (summer, 2.88 – 6.9 ‰, and winter, 2.88 – 7.8 ‰) are in the ranges of other studies (unpubl. data from Schell et al. 1998, Norderhaug et al. 2003, Schaal et al. 2010). Upwelled nitrate from the northeast Pacific Ocean has a $\delta^{15}N$ value between 5 and 7 ‰ (Miyake and Wada 1967). Under nutrient limited conditions, such as the nearing the end of a phytoplankton bloom, all nitrate would be incorporated in its entirety by phytoplankton and have a $\delta^{15}N$ value similar to the

source nitrate (Altabet and Francois 1994). This was visible in the present study during the summer as areas with no detectable nitrate and high chl *a* concentration had $\delta^{15}N$ values between 6 and 8 ‰ (Fig. 2.2 and 2.3). During the winter, the POM $\delta^{15}N$ values were expected to decrease because nitrate was not limiting and fractionation would have been maximized; however, this was not the case (Fig. 2.6, panel B). Phytoplankton $\delta^{15}N$ has been shown to vary among species and could be a result of cellular nitrogen metabolism, active transport across cell membranes or nitrate reductase activity, among others (Pennock et al. 1996 and references within). There is evidence that phytoplankton $\delta^{15}N$ may vary due to species composition (Montoya and McCarthy 1995) and distinctive cellular mechanisms for nitrogen metabolism and/or differences in intracellular storage of inorganic nitrogen (Dortch 1982, Dortch et al. 1984).

2.4.3 Spatial and temporal distribution of kelp-derived detritus

The results of this study indicate that the moderate to high KDD contribution to total and 20 – 63 μm POM year-round at all distances offshore (≥ 4 km; Fig. 2.8 and 2.9) of the otter-present region is likely due to the oceanographic conditions specific to this region that reduce the abundance of KDD, large confidence intervals in modeled KDD estimates due to similarity of source isotopes values and associated uncertainty, and high productivity of otter-absent region kelps. Summer growth and accumulation of kelp biomass is released into the environment through senescence and dissolved organic carbon (Lucas et al. 1981). Winter storms exacerbate the breakdown of kelp and release the biomass gained in the summer during the winter (Dayton 1985). Harris (2001) postulated that the water beyond the continental shelf off northern Vancouver Island was similar to shelf water due to the narrow (~5 km) continental shelf along the northern part of Vancouver Island. Wind driven upwelling filaments regularly form off Brooks Peninsula (just north of the otter-present region) during the summer and move a significant proportion of the nutrients, phytoplankton and zooplankton off the shelf into the pelagic environment (Forbes and Denman 1991). Moreover, when the north-flowing VICC meets the Brooks Peninsula it is deflected offshore, occasionally forming eddies (Thomson et al. 1989), and may possibly pull the nearshore KDD away from shore. Harris (2001) explained the SST gradient along the WCVI from their data, with lower temperatures off the otter-present region, as a result of the narrow shelf because upwelled water off the otter-present region has a shorter distance to travel and less time to warm before being transported off the shelf. This could have implications for water residence time over the shelf and upwelled POM depleted in ^{13}C being in the nearshore area depleting coastal POM $\delta^{13}C$ overall. Although KDD production may be relatively high in this region it is possible that the retention of this production in this region is reduced by regional oceanography.

In contrast, the KDD contribution to POM off of the otter-absent region was surprisingly high. During the summer, the nearshore contribution of KDD to POM rivalled or exceeded that of the otter-present and intermediate regions. This could be due to the POM $\delta^{13}C$ depleting environment present in the otter-present region described above, and/or to differences in the

dominant kelp species present in these regions. In particular, *Nereocystis* was markedly more abundant than *Macrocystis* in the otter-absent region (Martone and Markel, in prep). Furthermore, *Nereocystis* $\delta^{13}C$ values were more depleted than *Macrocystis* during the summer and winter in both the otter-present and otter-absent regions and more similar to phytoplankton isotope values. Similarity between the *Nereocystis* and phytoplankton values led to poor model performance and large uncertainty in modeled KDD contribution estimates and possibly to an overestimation of the contribution within the otter-absent region. This is further affected by relatively high uncertainty in kelp isotope values.

Overall, these results support the hypothesis that KDD in the otter-present region make a significant contribution to POM even at distances of up to 30 km from shore during summer and up to 10 km during winter. However, the maximum possible contribution was not as high as anticipated in an area with such large kelp forests, especially to total POM where one would expect large kelp fragments. Another possible explanation for this result is the differences among the regions with respect to kelp forest species composition because the presence or absence of sea otters influences kelp forest species composition and potentially alters the productivity of the kelp forest itself as annual species are succeeded by perennials after urchins are removed by sea otters (Duggins 1980). Annual kelps (ex. *Nereocystis*) dominate the otter-absent region and are more productive than perennial kelps (ex. *Macrocystis*) that are more prevalent in the otter-present region (Paine 2002). This difference between these two regions could lead to our results presented here as a highly productive annual kelp species in the otter-absent region could be overshadowing the difference in kelp abundance. It is also possible that the summer of 2009 was a highly productive season for kelp in the otter-absent region and that abundance was also greater; however, there is no data to support this hypothesis and year to year variation in abundance is thought to be minimal (L. Druehl, pers. comm.).

2.4.4 Summary

In this study it was found that phytoplankton $\delta^{13}C$ and $\delta^{15}N$ values varied with phytoplankton size fraction, physiological condition (i.e. blooming or not) and season. Similarly, kelp isotope values varied among species, regions, and seasons along the WCVI. These results support a growing number of studies demonstrating that kelp-derived production can be transported as much as 30 km from source kelp forests during the summer and up to 10 km during the winter. Surprisingly, this was also true for a region with relatively low kelp abundance, suggesting that there may be differences in kelp species' detrital and DOM production due to differences in kelp productivity among the regions. Differences among regions in kelp forest community composition with respect to the relative proportion of perennials and annuals may lead to the greater productivity of annual kelp species (i.e. the otter-absent region) outweighing the overall abundance of perennial dominated regions (i.e. the otter-present region). Factors such as regional differences in local oceanography and bathymetry may also be at play, reducing the abundance of local KDD productivity and dispersing it great distances.

Additionally, high uncertainty in kelp and phytoplankton isotope values leads to high uncertainty in kelp contribution modeled estimates which may obscure and limit the detection of possible differences in KDD contribution among regions. The KDD contribution to POM was generally higher during the summer; however, in areas where there were large differences between summer and winter, such as transect 4 in the otter-absent region, terrestrial input (i.e. freshwater) are likely to have been responsible. Kelp-derived allochthonous nutrient subsidies to offshore areas likely benefit consumers by providing a more year-round food source (Harrold et al. 1998), and potentially create more stable and biodiverse food webs with longer food chains (Moore et al. 2004).

Chapter 3: The relative dietary importance of kelp-derived detritus to plankton and benthic organisms off the west coast of Vancouver Island

3.1 Introduction

Identifying drivers of nearshore ecosystem productivity, diversity, and stability contribute to our understanding of food web, community and ecosystem ecology. Detritus, as an autochthonous and allochthonous subsidy, is an important bottom-up driver of trophic structure and food web dynamics by influencing energy, carbon and nutrient budgets (Vetter 1994, Polis et al. 1997 and references within). Generally, the biomass of benthic organisms is a function of local water column productivity (Polis et al. 1997 and references within); however, the flow of subsidies across habitats can create diverse webs where local productivity is low (Polis et al. 1997). The dispersal and concentration of this subsidy can be affected by local surface and bottom currents (Harrold et al. 1998), regional productivity, and the ratio of habitat perimeter to area (Polis and Hurd 1996).

Coastal marine consumers feed directly, or indirectly, on kelp- and/or phytoplankton-derived organic matter. Kelps (Phaeophycea: Order Laminariales) are large fleshy macroalgae that occupy low intertidal and shallow subtidal rocky reef habitats of temperate coastal marine ecosystems (Dayton 1985). Kelps contribute substantially to nearshore primary productivity (Mann 1973), with estimates ranging from 460 to 3000 g C m^{-2} yr^{-1} (Mann 1973, Coon 1982, Abdullah and Fredriksen 2004). In British Columbia, bull kelp (*Nereocystis luetkeana*) sporophytes can assimilate 1400 g C m^{-2} (Foreman 1984) and giant kelp (*Macrocystis pyrifera*) productivity estimates range from 460 to 1300 g C m^{-2} yr^{-1} (Mann 1973, Coon 1982, Druehl and Wheeler 1986). Most of the ocean-based photosynthesis occurs along coastal margins, which occupy only 0.1% of the total ocean area but phytoplankton productivity in upwelling regions is estimated to range from 200 to 973 g C m^{-2} yr^{-1} (Pauly and Christensen 1995, Hahm and Kim 2001). Phytoplankton concentrations along the WCVI reach a maximum during late spring to mid-summer when solar radiation is high and nutrients are readily available from winter mixing of the water column and upwelling (Parsons and Lalli 1988, Whitney et al. 1998). The relative contribution to particulate organic matter (POM) of these two main primary production sources (phytoplankton and macrophytes) can vary spatially and temporally (e.g. Kaehler et al. 2000, 2006, Hill et al. 2006); however, the year-round presence of kelp-derived detritus (KDD) could be important in food web dynamics, and system stability, and have considerable effects on trophic structure and biodiversity (Moore et al. 2004).

Kelp production is important to kelp associated communities (Kaehler et al. 2000) but it is also transported great distances from source populations as suspended POM (Kaehler et al. 2006, Hill et al. 2006). Kelp-derived carbon can be detected at great depths (Harrold et al. 1998) and is known to subsidize island and nearshore communities (Polis and Hurd 1996, Anderson and Polis 1998, Kaehler et al. 2000). However, some studies have called into question the

suitability of kelp as a food source because of high C:N ratios (Russell-Hunter 1970), high concentrations of secondary metabolites (Duggins and Eckman 1997), and structural rigidness (Padilla 1985). Conversely, bacterial degradation of KDD can lower C:N ratios and secondary metabolite content (Norderhaug et al. 2003), thereby increasing its nutritional quality. Relative to phytoplankton, kelp-derived carbon can contribute similarly or more to the diets of benthic invertebrate filter-feeders with estimates as high as 90% (Duggins et al. 1989, Bustamante and Branch 1996, Kaehler et al. 2000). More importantly, KDD can increase consumer growth rates by 2 to 5 fold, relative to pure phytoplankton diets (Duggins et al. 1989), and may have important consequences for population dynamics and ecosystem productivity (Moore et al. 2004 and references within).

Plant and animal tissues can be analysed to determine the relative abundance of a particular element's stable isotopes (usually carbon and nitrogen). Different groups of organisms have varying stable isotope ratios, which indicate a difference in the relative abundance of heavy vs. light isotopes (e.g., ^{13}C:^{12}C or $\delta^{13}C$). Higher relative abundance of ^{13}C is referred to as "enriched" and a lower relative abundance is referred to as "depleted". For this reason, food sources that have different isotopic values can be used to determine energy flow within food webs and to estimate an organism's specific diet (Bustamante and Branch 1996, Post 2002, Fry 2006). Carbon and nitrogen stable isotope analysis is a powerful tool in food web ecology used to quantify the incorporation of potential food sources (e.g. phytoplankton and kelp) into consumers and estimate the average trophic levels of consumers (DeNiro and Epstein 1977)(de Niro and Epstein 1976, Post 2002 and references within). In marine ecosystems, $\delta^{13}C$ values typically increase by 0 to 1 ‰ per trophic level (DeNiro and Epstein 1978, Rounick and Winterbourn 1986, Post 2002, McCutchan et al. 2003). Changes in $\delta^{15}N$ values with each trophic level transfer vary with tissue type (Mateo et al. 2008 and references within) and feeding mode (McCutchan et al. 2003). The average increase of $\delta^{15}N$ values with trophic level ranges from 1.4 ‰ for consumers of invertebrates (McCutchan et al. 2003) to 4 ‰ for filter-feeders (Bustamante and Branch 1996).

In this study, we investigated how contributions of KDD to size-fractionated plankton and benthic organisms vary spatially along a gradient of kelp abundance driven by recovering sea otter populations on the WCVI. The research questions were twofold: first, does KDD contribute proportionately more to the tissues of benthic invertebrates near and offshore in a region where kelp productivity is approximately 20-times higher as a result of sea otter recovery (Markel 2011)? Second, does the KDD contribution to benthic invertebrates vary between summer and winter due to this sea otter and kelp abundance gradient?

It is important to recognize the possibility of oceanographic factors influencing the spatial and temporal distribution and abundance of KDD, and therefore, its incorporation into regional food webs. Oceanographic factors, such as ocean currents, bathymetry and upwelling, may vary spatially and temporally (Thomson et al. 1989), which may interact with regional differences in

ecosystem perimeter to area ratios (Polis and Hurd 1996) leading to unanticipated results (Doak et al. 2008) and the region-specific decrease of KDD abundance.

Known regional kelp and phytoplankton isotope values ($\delta^{13}C$ and $\delta^{15}N$) were used in Bayesian mixing models (MixSIR) to quantify the percent relative contribution of kelp- versus phytoplankton-derived detritus to plankton and benthic invertebrates. It was predicted that nearshore and offshore organisms in the high kelp productivity region would have higher proportions of KDD, in comparison to a region where otters are absent and kelp productivity is low, during summer and winter.

3.2 Materials & Methods

3.2.1 Study system

This research was conducted along the WCVI, British Columbia, Canada (Fig. 3.1), situated at the northern end of the California Current Large Marine Ecosystem (Sherman and Alexander 1986). This coastline is characterized by complex oceanographic processes. Seawater over the outer shelf (seaward of the 100 m depth contour) flows pole-ward during the winter but changes direction from north-westward to south-eastward during the spring with the seasonal onset of coastal upwelling, and the reverses again in the fall (Thomson 1981, Shanks and Eckert 2005). The flow over the inner continental shelf (shoreward of the 100 m depth contour) is dominated by the buoyancy driven Vancouver Island Coastal Current (VICC). This current flows pole-ward year round but can be reversed in the upper 50 m by strong northwest winds that occur during strong upwelling favourable conditions in summer or during cold outflow conditions during winter (Thomson et al. 1989).

Sea otters are voracious consumers and exert strong top-down control of many benthic invertebrates, most notably sea urchins (*Strongylocentrotus* sp.). In the absence of sea otters, hyper-abundant urchin populations dramatically reduce macroalgal populations and result in widespread urchin “barrens” (Pearse and Hines 1979). In the presence of sea otters and absence of sea urchins, expansive kelp forests support diverse and productive ecosystems (Estes and Palmisano 1974, Dayton 1985, Steneck et al. 2002). As a result of the North Pacific maritime fur trade, sea otters were extirpated from the coast of British Columbia by 1929 (Cowan and Guiguet 1960). However, between 1969 and 1972 eighty-nine animals were reintroduced to the northwest coast of Vancouver Island from western Alaska (Bigg and MacAskie 1978). Subsequently, this population has increased rapidly and expanded its range north and southwards along the WCVI (Nichol et al. 2005, 2009). At the time of sampling, sea otters were distributed from the northern tip of Vancouver Island southward to an area just south of Clayoquot Sound (Fig. 3.1) and have dramatically increased kelp populations along this coastline (Markel 2011, Watson and Estes 2011).

Figure 3.1. Sampling regions along the west coast of Vancouver Island (WCVI). The red lines represent the summer transects (0 – 30 km). During the winter, sampling was only to 10 km from the kelp forest in the otter-present and otter-absent regions, and only at 4 and 8 km in the otter-intermediate region. Sampling transects indicated by T1, T2, etc.

3.2.2 Experimental Design

Three research cruises were conducted off the west coast of Vancouver Island, British Columbia between July 2009 and July 2010. The study region includes three large Sounds that occur along this coastline that are characterized by large differences in kelp biomass corresponding to sea otter occupation time (Markel 2011, Watson and Estes 2011). A 'space-for-time substitution' approach (Pickett 1989) was used to take advantage of sea otter reintroduction and range expansion on the WCVI. Kyuquot Sound (Fig. 3.1), in the north (nearest to where sea otters were first reintroduced and re-established), has the highest kelp biomass and is hereafter referred to as 'otter-present'. Clayoquot Sound is hereafter referred to as 'otter-intermediate', and Barkley Sound with the least kelp biomass (Martone and Markel, in prep) is referred to as 'otter-absent'. The otter-present and otter-absent regions were approximately 170 km apart. Two transects (approximately 10 to 12 km apart) originated within kelp forests at the mouth of each sound and ran perpendicular to the coastline with sampling at 0, 0.5, 1, 2, 4, 10 and 30 km from the kelp forest. In the 'otter-absent' and 'otter-present' regions summer sampling occurred

between July 18th and 23rd, 2009; summer sampling off the otter-intermediate region was conducted between July 27th and July 28th, 2010. Winter sampling only took place off the otter-present and otter-absent regions between January 23rd and 31st, 2010 due to inclement weather.

3.2.3 Size-fractionated plankton stable isotopes

At each station, surface size-fractionated plankton: 63 – 125 µm, 125 – 250 µm, 250 – 500 µm, and 500 – 2000 µm were collected. Surface tows were performed by towing a 63 µm net horizontally for 5 to 10 minutes at approximately 1 knot. All samples were size-fractionated and frozen in a -20 ºC freezer at sea. Some samples did not have all size-fractions analyzed due to lack of sample volume.

In the laboratory, all samples were thawed, randomly sub-sampled in triplicates to identify and enumerate major plankton groups within each size fraction. Samples were then dried in a Fisher Scientific oven at 50ºC for at least 24 hours, ground into a powder and packaged into 5x8 mm tin capsules for stable isotope analysis. KDD was visible microscopically, especially in the 63 – 125 µm and 125 – 250 µm size fractions, but was difficult to quantify and is therefore not included.

All isotope analyses were conducted at IsoEnvironmental in Grahamstown, South Africa. Abundances of naturally occurring carbon and nitrogen stable isotopes were determined on either a Europa Scientific Integra IRMS or Europa Scientific 20-20 IRMS linked to an ANCA SL Elemental Analyser. Beet sugar and ammonium sulphate were used as internal standards, calibrated against several International Atomic Energy Agency (IAEA) reference materials. Results are expressed in the standard delta notation, as $X = [(R_{sample} / R_{standard}) - 1] \times 1000$, where X = element in question and R = ratio of the heavy over the light isotope. Repeated analyses of homogeneous material yielded a $\delta^{13}C$ standard deviation of 0.11 ‰ and a $\delta^{15}N$ standard deviation of 0.16 ‰.

3.2.4 Benthic organism stable isotopes

Benthic dredging was performed with a 60 cm by 30 cm dredge towed along the bottom for 5 to 10 minutes at approximately at a speed of 1.5 to 2 knots. This was done at all stations except from within the kelp forests (0 km) and at some 30 km stations that were too deep to sample. Samples were immediately frozen in a -20 ^{o}C freezer. In the laboratory, benthic samples were thawed, identified to lowest possible level, and rinsed with distilled water. The tissues of the most common species/groups (muscle or, whole body minus the shell) were dried for at least 24 hours at 50ºC. Dried samples were ground with a mortar and pestle or digital amalgamator, and packaged in tin capsules for carbon and nitrogen stable isotope analysis. Benthic samples from within the kelp forests were collected using SCUBA during the summer of 2009.

To test for acidification effects on isotope values, 7 hermit crabs (Infraorder: Anomura) were acidified with 0.1 M HCl from an eye-dropper, while the remaining 61 remained untreated. The number of drops varied from 5 to 17 and was applied until CO_2 gas stopped being released, i.e. stopped bubbling (Jacob et al. 2005). The sample was then re-dried without rinsing to minimize the loss of DOM and ground into a powder again (Fry 1988, Cloern et al. 2002).

To test for lipid extraction (i.e. defatting) effects on *Astraea gibberosa* (red turban snail) stable isotope values, five whole snails were split in half and one half was defatted, and the other remained untreated, following the method of Bligh and Dyer (1959) and then lightly rinsed with distilled water on 0.7 µm Whatman GF/F filters with light suction. Samples were re-dried and ground into a powder. Additionally, C:N ratios were used to check for excess lipids in both cases.

3.2.5 Data analyses

Size-fractionated plankton: Linear regression was used to assess the relationship between distance from the kelp forest and plankton $\delta^{13}C$ values.

Benthic organisms: Where assumptions were met, paired t-tests were used to test for effects of acidification and lipid extraction of benthic organisms. In cases of non-normality or heterogeneous variance, Wilcoxon Matched pairs tests were used. Linear regression was used to assess relationships between stable isotope values and organism size (length or width). All statistical analyses were performed in R (R Core Development Team. 2011).

MixSIR: We used the isotope mixing model program MixSIR (version 1.0, Semmens and Moore 2008) to determine percent KDD contribution to plankton and benthic organisms. MixSIR is a graphical user interface that performs Bayesian analysis of stable isotope mixing models using Hilborn sampling-importance-resampling (SIR) algorithm (Rubin 1988). The benefits of a Bayesian approach to stable isotope mixing models include: 1) accounting for uncertainty in source stable isotope values, 2) accounting for uncertainty in the estimates of source contributions as there is underlying uncertainty in the mixture and source isotope values, 3) determining a unique solution when more than two sources are present (Moore and Semmens 2008, Semmens et al. 2009). Outputs of the program include a median percent contribution of all sources included and their respective 95% confidence intervals. The degree of overlap between confidence intervals defines the probability that two estimates are the same. Source $\delta^{13}C$ fractionation and fractionation standard deviations were set to 0 ‰ for summer and winter plankton. Source $\delta^{15}N$ fractionation and fractionation standard deviations were set to 1.47 ± 0.39 ‰, while for winter it was 2.32 ± 0.46 ‰. For other consumers the values used were 0.5 ± 0.13 ‰ for $\delta^{13}C$ and 2.2 ± 0.3 ‰ (for primary consumers), 2.13 ± 0.18 ‰ (for omnivores and detritivores) and 3.3 ± 0.26 ‰ (for higher consumers) for $\delta^{15}N$ (McCutchan et al. 2003). These values will be justified in the 'Results' section.

3.3 Results

3.3.1 Kelp and phytoplankton isotope values

Kelp and phytoplankton isotope values used in MixSIR, to determine percent contributions of KDD, were reported in Chapter 1 (Table 2.3 and 2.4, respectively).

3.3.2 Surface plankton carbon stable isotopes

***Summer*:** Phytoplankton species composed the largest proportional abundance of the 63 – 125 µm (0.85) plankton surface tows and 0.35 of the 125 – 250 µm fraction. Different growth stages of copepods constituted the largest proportional abundance of plankton > 125 µm with proportions of 0.39, 0.45, and 0.47 for the 125 – 250 µm, 250 – 500 µm, and 500 – 2000 µm size fractions, respectively (Fig. 3.4, Table B3). Cladocerans made the second largest contributions to both the 250 – 500 µm (0.35) and the 500 – 2000 µm (0.17) fractions. Decapods were the third largest contributor to the 500 – 2000 µm plankton (0.14).

For a particular size fraction, among region differences in surface plankton $\delta^{13}C$ values did not exist. None of the regions had within region differences among size fractions with respect to $\delta^{13}C$ values. The size fraction means ranged from -18.72 ‰ for 250 – 500 µm plankton to -19.09 ‰ for 125 – 250 µm plankton in the otter-present region. In the otter-intermediate region the plankton $\delta^{13}C$ means ranged from -18.07 ‰ for 63 – 125 µm plankton to -19.20 ‰ for 250 – 500 µm plankton. The otter-absent region ranged from -18.26 ‰ for the 63 – 125 µm fraction to -20.23 ‰ for the 250 – 500 µm fraction. With all three regions combined, there were differences among size fractions. The 63 – 125 µm size fraction was more enriched than all other size fractions ($df = 4$, $F = 12$, $p < 0.05$ in all cases) which is likely due to the high concentration of enriched KDD in the 63 – 125 µm fraction that was seen microscopically. All size fraction means and standard deviations are available in Table B5.

The 125 – 250 µm fraction along transect 2 in the otter-present region had decreasing $\delta^{13}C$ values with distance from the kelp forest ($R^2 = 0.73$, $p = 0.03$). Along transect 6 in the otter-intermediate region the 250 – 500 µm fraction had decreasing $\delta^{13}C$ values with distance ($R^2 = 0.99$, $p = 0.06$). Transect 4 in the otter-absent region had decreasing $\delta^{13}C$ values with distance for three size fractions as well (63 – 125 µm: $R^2 = 0.87$, $p = 0.007$; 125 – 250 µm: $R^2 = 0.97$, $p<0.001$; 250 – 500 µm: $R^2 = 0.59$, $p = 0.04$). Including only samples with sufficient data, 6 out of 20 samples showed decreasing $\delta^{13}C$ values with distance from the kelp forest.

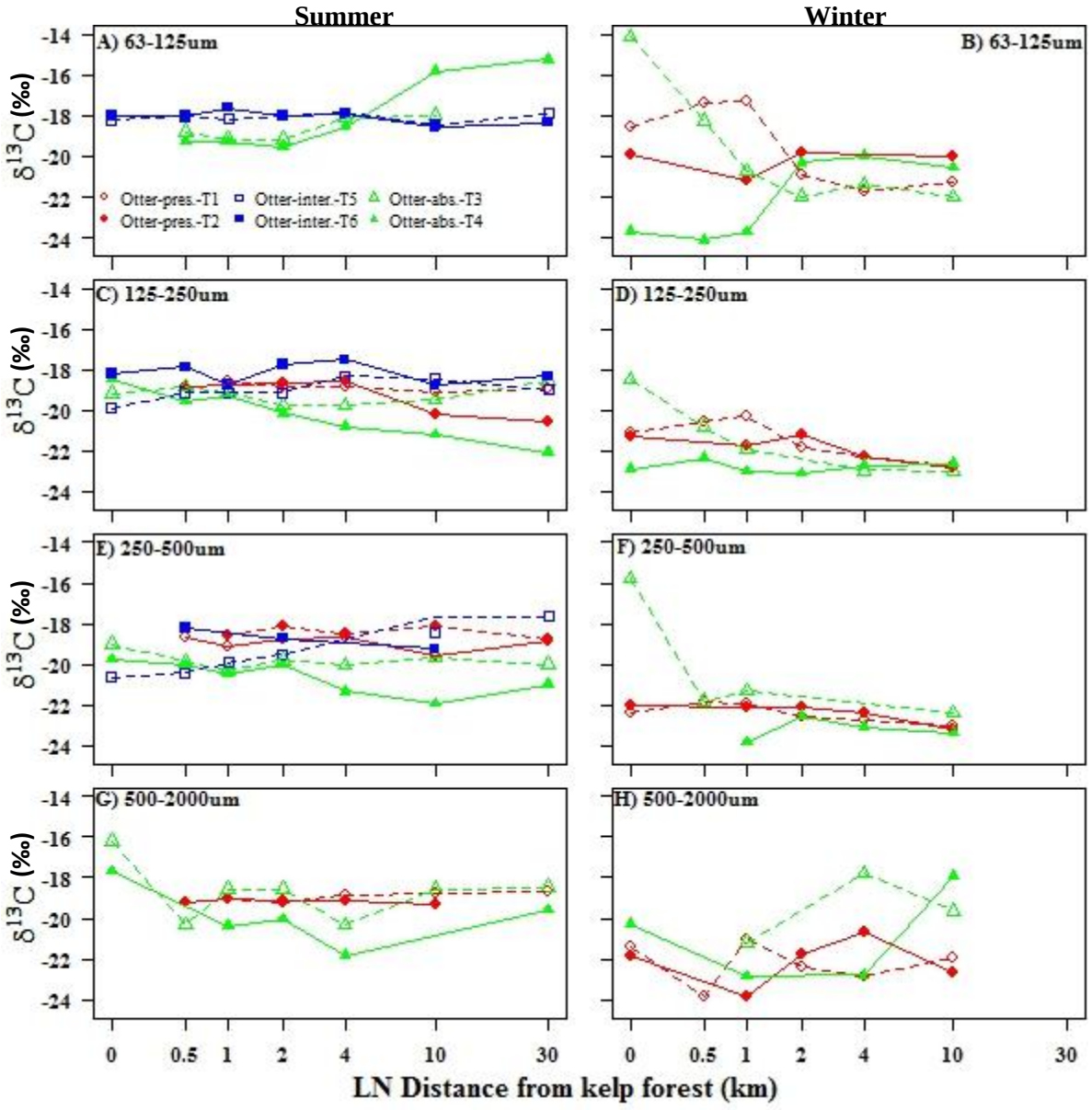

Figure 3.2. Summer and winter $\delta^{13}C$ (‰) for 63 – 125 µm, 125 – 250 µm, 250 – 500 µm, and 500 – 2000 µm zooplankton. Each point represents a single sample (n=1).

***Winter*:** Proportionally, phytoplankton species made the largest contribution to the 63 – 125 µm (0.82) and the 125 – 250 µm (0.54) surface tows. Copepods were the second most prevalent in the 125 – 250 µm fraction at 0.37. They constituted almost the entire 250 – 500 µm zooplankton with a proportion of 0.94 and were the most prevalent in the 500 – 2000 µm fraction (0.75). Veliger larvae made a minor contribution to the 63 – 125 µm fraction at 0.15 and less so to the 125 – 250 µm tow (0.07). Decapod and fish larvae constituted 0.1 and 0.08 of the 500 – 2000 µm plankton, respectively (Fig. 3.5, Table B4).

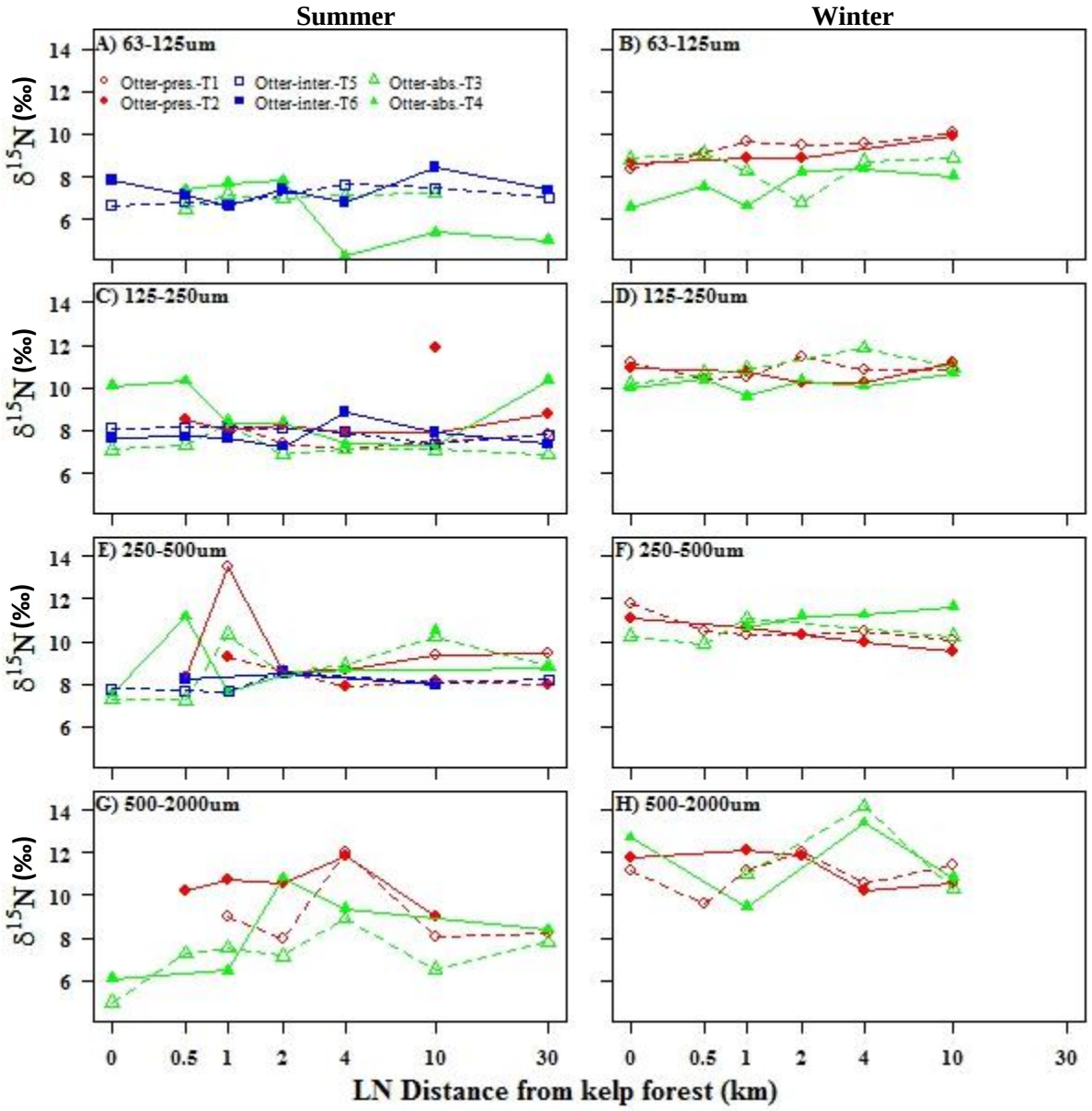

Figure 3.3. Summer and winter $\delta^{15}N$ (‰) for 63 – 125 µm, 125 – 250 µm, 250 – 500 µm, and 500 – 2000 µm plankton. Each point represents a single sample (n=1).

There were no differences in carbon enrichment for any size fractions among the three regions and there were no within region differences among the plankton size fractions. Furthermore, when otter-present and absent regions were combined there were no differences with respect to $\delta^{13}C$ values among size fractions (otter-intermediate region was not sampled). The otter-absent region plankton mean $\delta^{13}C$ values ranged from -19.80 ‰ for 63 – 125 µm plankton to -22.39 ‰ for the 250 – 500 µm fraction. The otter-absent region plankton means

ranged from -19.76 ‰ for the 500 – 2000 µm plankton to -22.20 ‰ for 125 – 250 µm plankton. When all three regions combined, there were no differences in size fraction $\delta^{13}C$ values. All size fraction means and standard deviations are available in Table B5.

The 125 – 250 µm plankton fraction along transect 1 in the otter-present region had significantly decreasing $\delta^{13}C$ values with distance from the kelp forest ($R^2 = 0.68$, $p = 0.04$). The 63 – 125 µm fraction along transect 3 in the otter-absent region also had decreasing $\delta^{13}C$ values with distance from the kelp forest ($R^2 = 0.71$, $p = 0.04$), while transect 4 within this same region had was more enriched in ^{13}C with distance ($R^2 = 0.67$, $p = 0.047$). The 125 – 250 µm plankton along transect 3 had decreasing $\delta^{13}C$ values ($R^2 = 0.81$, $p = 0.04$).

***Summer-Winter Comparison*:** Phytoplankton proportions remained constant within size fractions between summer and winter. Copepods increased during the winter in the 250 – 500 µm and 500 – 2000 µm fractions by 0.49 and 0.28, respectively. Cladocerans were not present during the winter. From summer to winter, barnacle larvae decreased in all fractions, while veliger larvae increased during the winter in the 63 – 125 µm and 125 – 250 µm fractions. Unidentified egg sacs were only found during the summer and decapods remained constant from summer to winter in the 500 – 2000 µm plankton.

Between season comparisons of the otter-present region plankton size fractions revealed all summer fractions being enriched relative to the winter (125 – 250 µm ($W = 120$, $p < 0.001$), 250 – 500 µm ($t = 20.1$, $df = 20$, $p < 0.001$), 500 – 2000 µm ($W = 110$, $p < 0.001$). The otter-absent region comparisons revealed that the 63 – 125 µm ($W = 114$, $p < 0.01$), 125 – 250 µm ($W = 151$, $p < 0.01$) and 250 – 500 µm ($W = 95$, $p < 0.01$) size fractions were all enriched in ^{13}C during the summer compared to the winter.

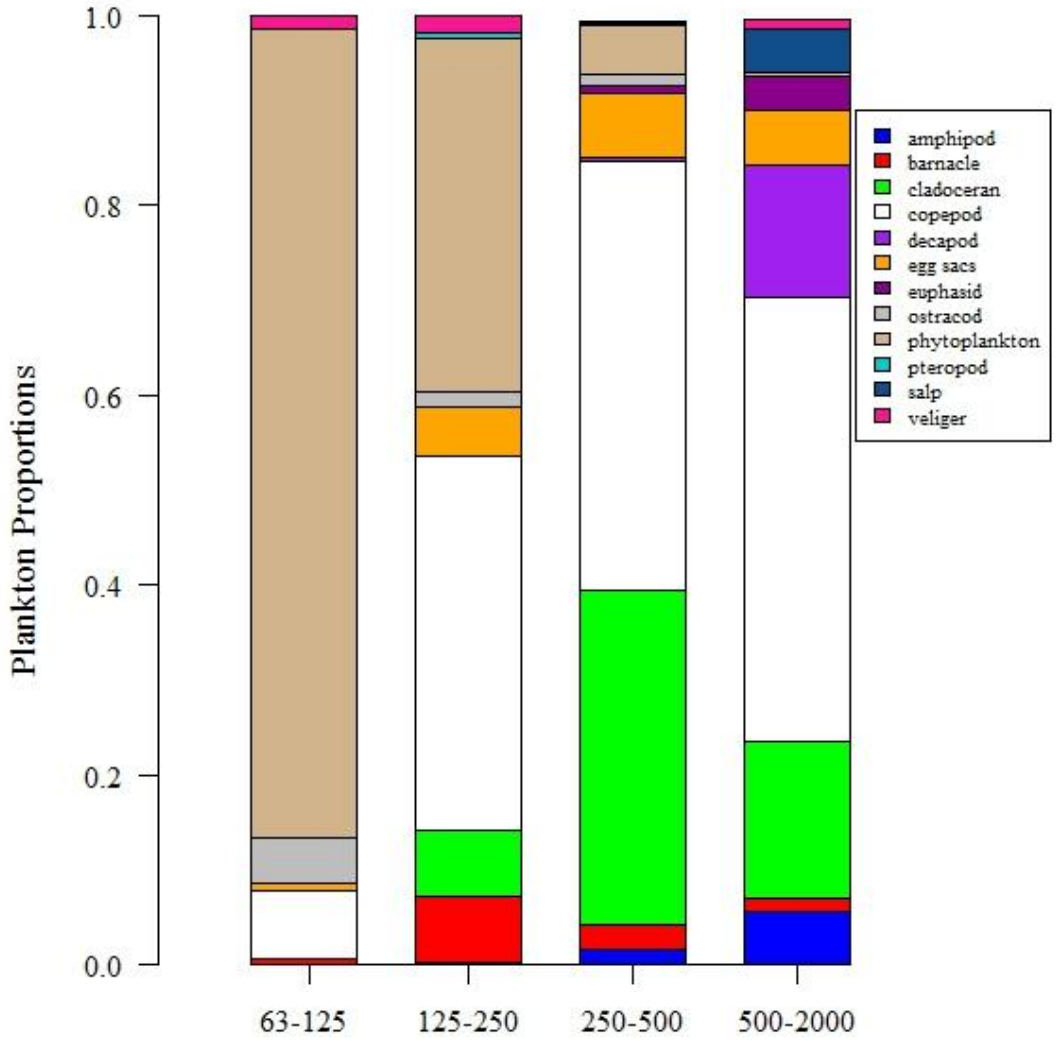

Figure 3.4. Summer proportional plankton by size fraction. Plankton groups that constituted < 0.5 % for all size fractions were removed. Kelp-derived and other detritus were not enumerated.

3.3.3 Surface plankton nitrogen stable isotopes

***Summer*:** With respect to plankton $\delta^{15}N$ values, the 500 – 2000 µm size fraction was more enriched in the otter-present region compared to the otter-absent region ($p = 0.049$). The otter-present region's mean $\delta^{15}N$ values ranged from 8.32 ‰ for 125 – 250 µm to 9.75 ‰ for 500 – 2000 µm plankton. The otter-intermediate region's mean values ranged from 7.23 ‰ for 63 – 125 µm plankton to 8.08 ‰ for the 250 – 500 µm fraction. The otter-absent region ranged from 6.59 ‰ for the 63 – 125 µm fraction to 8.86 ‰ for the 250 – 500 µm fraction. There were no significant within region $\delta^{15}N$ differences among the plankton size fractions. When all three regions were combined, the 63 – 125 µm was enriched in ^{15}N when compared to all other size

fractions (df = 4, F = 12, $p < 0.001$ in all cases), but no other fractions were different. All size fraction means and standard deviations are available in Table B5.

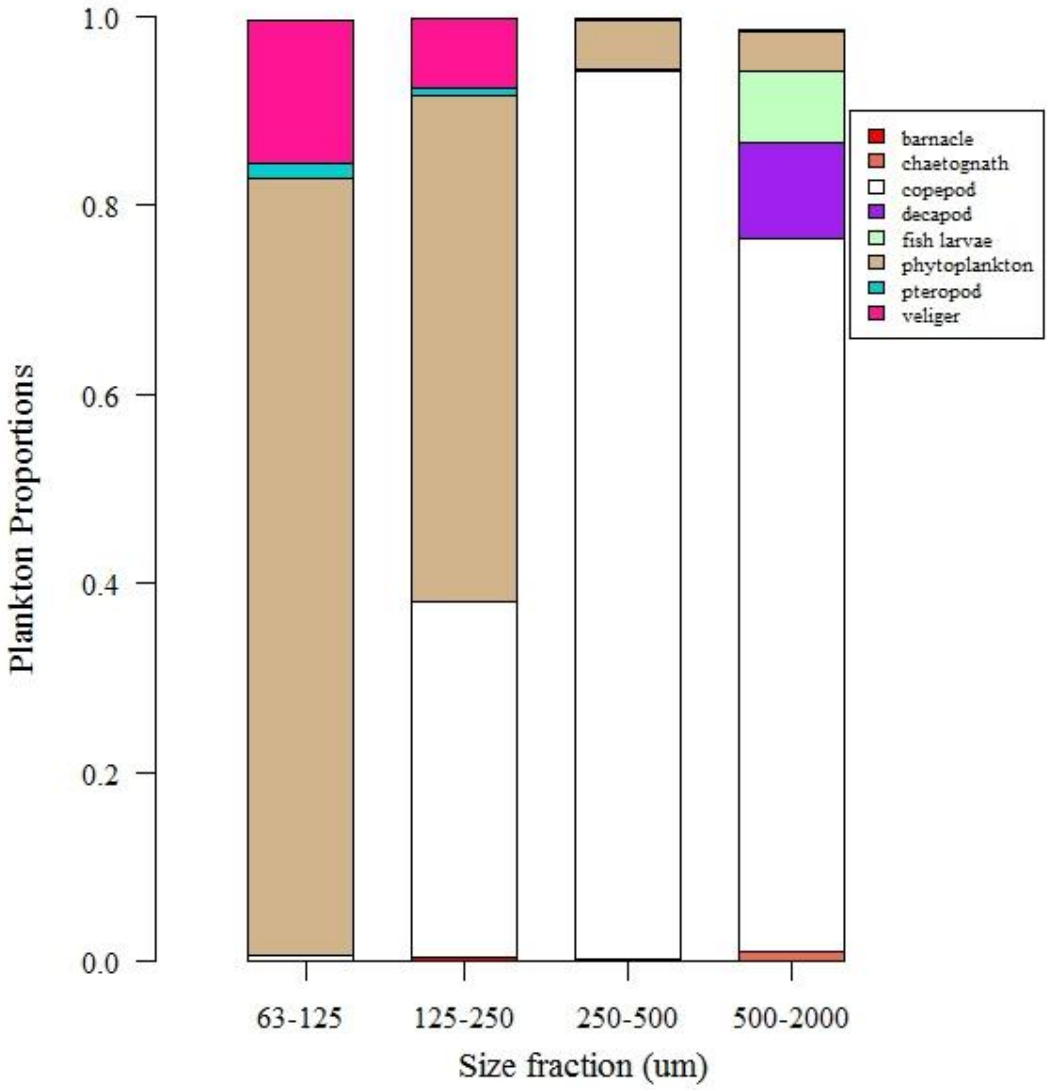

Figure 3.5. Winter proportional plankton by size fraction. Plankton groups that constituted < 0.5 % for all size fractions were removed. Kelp-derived and other detritus were not enumerated.

***Winter*:** During the winter there were no significant differences in plankton $\delta^{15}N$ values among the regions. The otter present region 63 – 125 µm plankton was depleted relative to the 125 – 250 µm and 500 – 2000 µm fractions ($p = 0.04$ and $p < 0.01$, respectively). There were no other size fraction differences within this region. Within the otter-absent region, the 63 – 125 µm fraction was depleted in ^{15}N when compared to all other fractions (df = 4, F = 32, $p < 0.001$ in all cases);

however, no other size fractions were different. The otter-absent region's plankton mean $\delta^{15}N$ values ranged from 9.25 ‰ for the 63 – 125 µm fraction to 11.12 ‰ for the 500 – 2000 µm fraction while the otter-absent region's values ranged from 7.99 ‰ for the 63 – 125 µm plankton to 11.69 ‰ for the 500 – 2000 µm fraction. All size fraction means and standard deviations are available in Table B5.

Summer-Winter Comparison: During the winter in the otter-present region, all plankton $\delta^{15}N$ values were enriched when compared to the same size fraction during the summer (125 – 250 µm: $W = 11$, $p < 0.001$; 250 – 500 µm: $W = 11$, $p < 0.001$; 500 – 2000 µm: $t = -2.6$, $df = 13$, $p = 0.02$). This was also true for the otter-absent region (63 – 125 µm: $t = -3.2$, $df = 19$, $p < 0.01$; 125 – 250 µm: $W = 12$, $p < 0.01$; 250 – 500 µm: $t = -4.8$, $df = 20$, $p < 0.001$; 500 – 2000 µm: $t = 1.3$, $df = 10$, $p < 0.001$).

3.3.4 Trophic level isotope fractionation

Although summer 63 – 125 µm plankton $\delta^{13}C$ was more enriched than the 125 – 250 µm, 250 – 500 µm and the 500 – 2000 µm fractions, there was no pattern or step-wise enrichment with size fraction (Fig 3.6). The mean ^{13}C fractionation (± SD) was -0.34 ± 1.51 ‰ (i.e. values were more depleted with increasing trophic level) between 63 – 125 µm and these two fractions. There was no difference among winter plankton $\delta^{13}C$ fractions. Considering these factors, source $\delta^{13}C$ fractionation and standard deviation were '0' for summer and winter.

Because the summer and winter plankton $\delta^{15}N$ values for the 63 – 125 µm size fraction was depleted when compared to all larger size fractions, and no other fractions were significantly different, a mean fractionation (± SD) for each season was calculated by subtracting the mean of each fraction with the 63 – 125 µm within its respective season (Fig. 3.6). For summer plankton this value was 1.47 ± 0.39 ‰, while for winter it was 2.32 ± 0.46 ‰.

For benthic organisms' isotope fractionation with trophic level, values from McCutchan et al (2003) were used as this work applied to marine organisms, and had separate values for herbivores, organisms raised on invertebrates, etc. ($\delta^{13}C$: 0.5 ± 0.13 ‰; herbivores - $\delta^{15}N$: 2.2 ± 0.3 ‰, raised on inverts - $\delta^{15}N$: 1.4 ± 0.21 ‰).

3.3.5 Kelp-derived detritus incorporated by plankton

Summer: During the summer in the otter-present region, the median KDD contributions to plankton were from 21 to 81 % for all size fractions combined. Individual size fractions revealed that the median KDD contributions were from 21 to 67 % (4 to 80 %, maximum contribution range; Fig. 3.7 panel B) of the 125 – 250 µm fraction. Median KDD contributions to this fraction remained high and constant with distance from the kelp forest. The two largest fractions were similar in this regard with the exception of a few distances where the median KDD contribution

was much higher, such as 1 km along the 250 – 500 µm fraction. For example, for the 250 – 500 µm fraction at 1 km the median contribution was 81 % (71 to 89 % C.I.; Fig. 3.7, panel C). Within a size fraction there was large overlap in confidence intervals with distance and between transects at the same distance except for the 500 – 2000 µm fraction where there was greater variability with distance but the two transects were generally similar (Fig. 3.7, panel D).

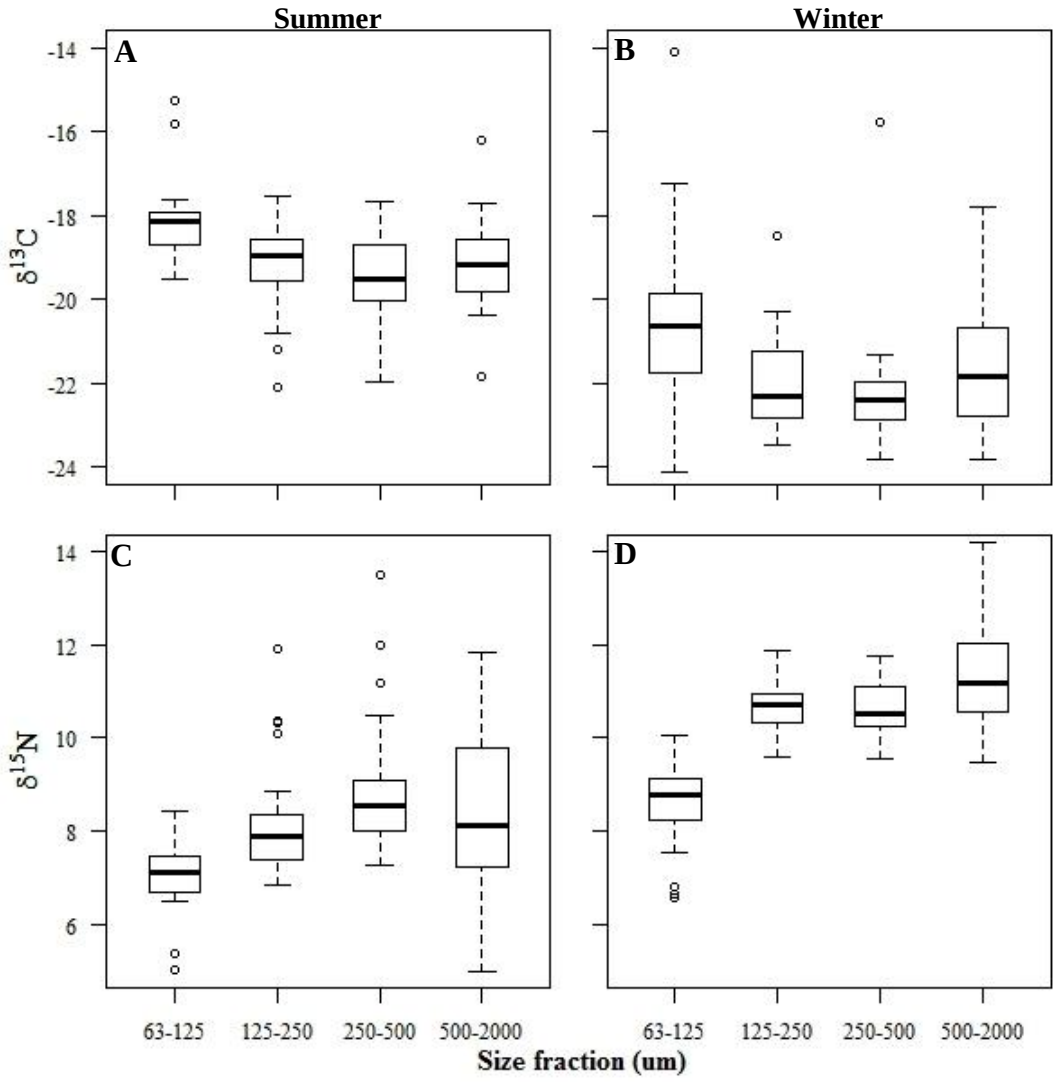

Figure 3.6. Summer and winter $\delta^{13}C$ and $\delta^{15}N$ values (‰) of size-fractionated surface plankton from all three regions combined. Summer and winter 63 – 125 µm plankton was significantly depleted in ^{15}N compared to larger fractions within their respective seasons.

In the otter-intermediate region, median KDD contributions to plankton were between 16 and 55 % (2 to 70 %, maximum contribution range) for all size fractions combined. For 63 – 125

µm plankton the median KDD percent contributions were from 47 to 55 % (31 to 70 %, maximum possible C.I. range) and remained moderately high and constant with distance from the kelp forest (Fig. 3.7, panel A). Median KDD contributions to 125 – 250 µm plankton were slightly lower (25 to 55 %, with a maximum possible contribution range of 5 to 70 %) and even lower for the 250 – 500 µm plankton with a median contribution range of 16 to 52 % (2 to 68 %, maximum possible contribution range). Samples greater than 500 µm were not collected for this region.

In the otter-absent region, median percent KDD contribution to plankton was highly variable with distance and between transects for all size fractions of plankton. For all size fractions median contributions were low (16 %, C.I. of 1 to 44 %; Fig. 3.7, panel D) to very high (81 %, C.I. of 72 to 90 %; Fig. 3.7, panel A). Median contributions to the 63 – 125 µm fraction were moderate to very high and increased with distance along transect 4. Median contributions to 125 – 250 µm plankton had large uncertainty at a number of distances (Fig. 3.7, panel B). This led to large overlap in percent contributions between the two transects for this size fraction. Transect 1 medians decreased with distance to 10 km and then increased dramatically at 30 km from the kelp forest. Transect 2 medians were much more consistently moderate but the large confidence intervals previously mentioned are masking any possible trends. Large confidence intervals were also present for the 250 – 500 and 500 – 2000 µm plankton. This led to a large range of possible contributions across the region for these two size fractions. The 250 – 500 µm fraction median KDD contributions were from 16 to 65 % (2 to 78 %, maximum contribution range). The 500 – 2000 µm fraction had even larger possible KDD contribution with medians from 16 to 80 % (1 to 89 %, maximum possible contribution).

Winter: In the otter-present region, the median KDD contributions to plankton were from 4 to 68 % for all size fractions combined. Individual size fractions revealed that the median KDD contributions were from 41 to 68 % (18 to 83 %, maximum contribution range; Fig. 3.8 panel A) of the 63 – 125 µm fraction. Median KDD contributions to this fraction remained high and constant with distance from the kelp forest with large overlap in confidence intervals with distance and between transects. For the 125 – 250 µm plankton median KDD contributions decreased moderately with distance and had relatively low overlap in confidence intervals between 0 and 10 km, indicating that there is a low probability that the contributions were similar between these two distances (Fig. 3.8, panel B). The 250 – 500 µm had smaller medians relatively to the two smaller fractions and only decreased slightly with distance within the fraction (panel C). Median percent KDD contributions for this fraction were from 13 to 29 % (2 to 61 %, maximum possible contribution range). The 500 – 2000 µm fraction was highly variable with distance and had confidence intervals that did not overlap at certain distances (e.g. 0.5 and 1 km along transect 1; panel D). This fraction's median KDD contributions were from 6 to 41 % (1 to 65 %, maximum range of confidence intervals).

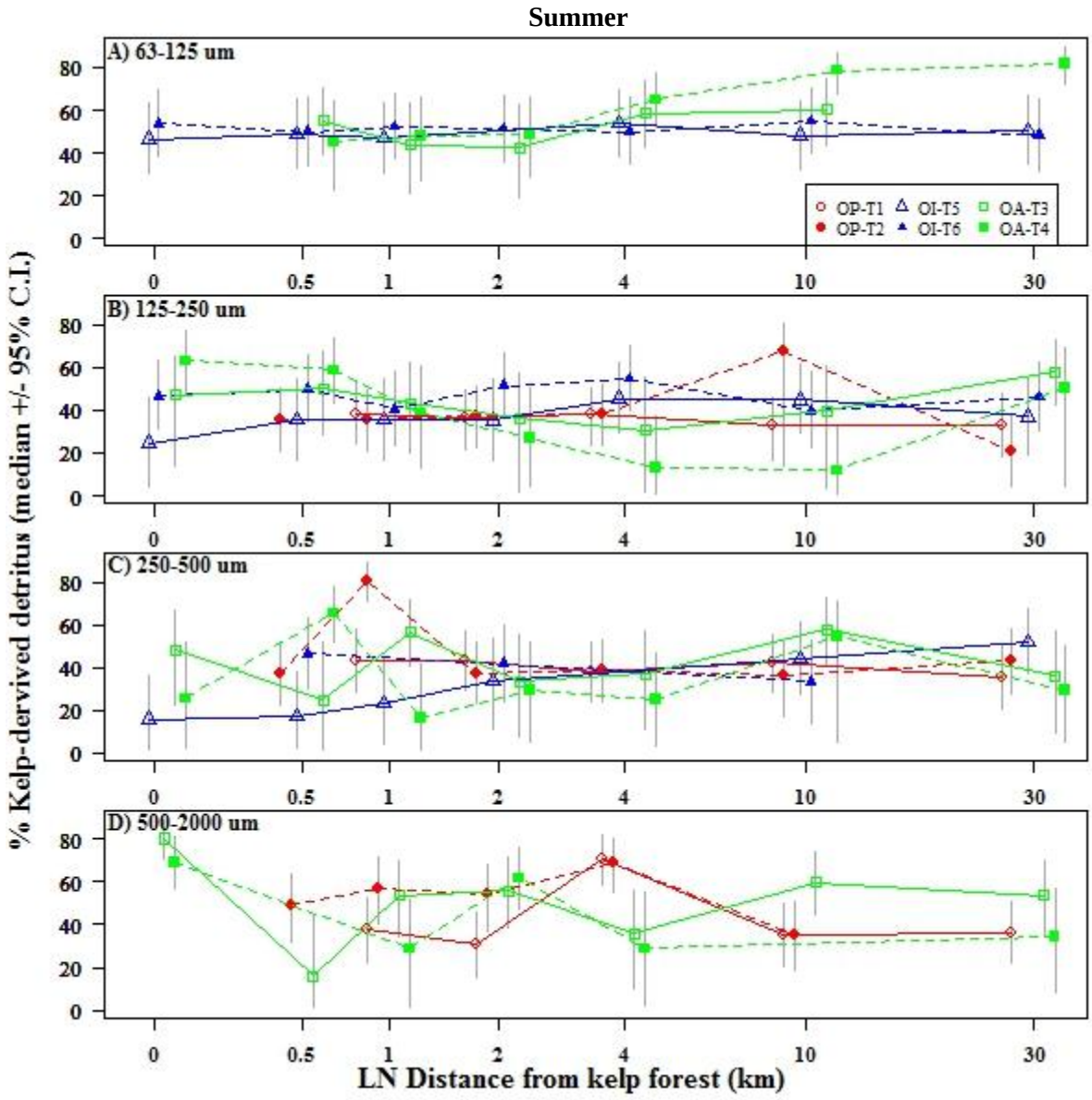

Figure 3.7. Summer percent kelp-derived detritus contribution (median ± 95 % C.I.) to surface 63 – 125 µm, 125 – 250 µm, 250 – 500 µm, and 500 – 2000 µm plankton for all three regions. Each point is a single sample (n = 1). For display purposes points are jittered horizontally.

During the winter in the otter-absent region, KDD median contributions to plankton were from 4 to 80 % (0 to 91 %, maximum contribution confidence interval range) for all size fractions combined. The 63 – 125 µm fraction had a strong contrast with respect to KDD contribution between transects as transect 3 medians decreased with distance and transect 4 medians increased (Fig. 3.8, panel A). Transect 3 KDD contribution within the kelp forest (0 km) was 80 % (68 to 91 % C.I.) and decreased to 27 % (9 to 61 % C.I.) 10 km from the kelp forest. Transect 4 remained had a low contribution from 0 to 1 km (~ 6 % with a 0 to 21 % C.I.). At 10 km from the kelp forest the median KDD contribution was 47 % (28 to 66 % C.I.). Again, for 125 – 250 µm plankton the median KDD contribution decreased with distance and had no overlap in confidence intervals between 0 and 10 km (panel B). Transect 4 KDD contributions remained low and constant with distance. The 250 – 500 µm plankton showed a similar decline in contribution with distance and had a sharp decline between 0 and 0.5 km and confidence intervals did not overlap (panel C). At 0 km the median contribution was 73 % (59 to 86 % C.I.) and decreased to 20 % (6 to 44 % C.I.). The 500 – 2000 µm fraction had no trends and was highly variable with distance. The median KDD contributions were from 15 to 79 % (3 to 91 %, maximum contribution confidence interval; panel D).

3.3.6 Kelp-derived detritus incorporated by benthic organisms

Astraea: Paired defatted and untreated *Astraea gibberosa* samples showed no differences in $\delta^{13}C$ and $\delta^{15}N$ values. Further analyses were only on the untreated samples.

Astraea mean lengths (± SE) differed among regions ($df = 2$, $F = 29$, $p < 0.001$). The otter-present region individuals (3.43 ± 0.19 cm) were smaller in size compared to the otter-intermediate (5.4 ± 0.15 cm) and absent regions (5.59 ± 0.23 cm; $p < 0.001$ and $p < 0.001$, respectively). When all regions were pooled, *Astraea* $\delta^{13}C$ and $\delta^{15}N$ values increased with length ($R^2 = 0.47$, $p < 0.001$, and $R^2 = 0.11$, $p = 0.03$).

There was considerable within region $\delta^{13}C$ variation among sampling sites ($df = 7$, $F = 9$, $p < 0.001$) which didn't allow samples from within the same region to be pooled for modeled KDD contribution estimates as this led to bimodal posterior probability distributions. This variation did not exist for $\delta^{15}N$ values.

Percent KDD incorporated by *Astraea* showed among and within region variation with large differences in percent KDD contribution between the otter-absent region and the otter-intermediate regions. *Astraea* collected in the otter-absent region were composed of 0 to 27 % KDD (range of medians); however, there was large variation within this region as samples from Kamils Island were composed of 27 % (22 to 32 % C.I.) KDD while the other

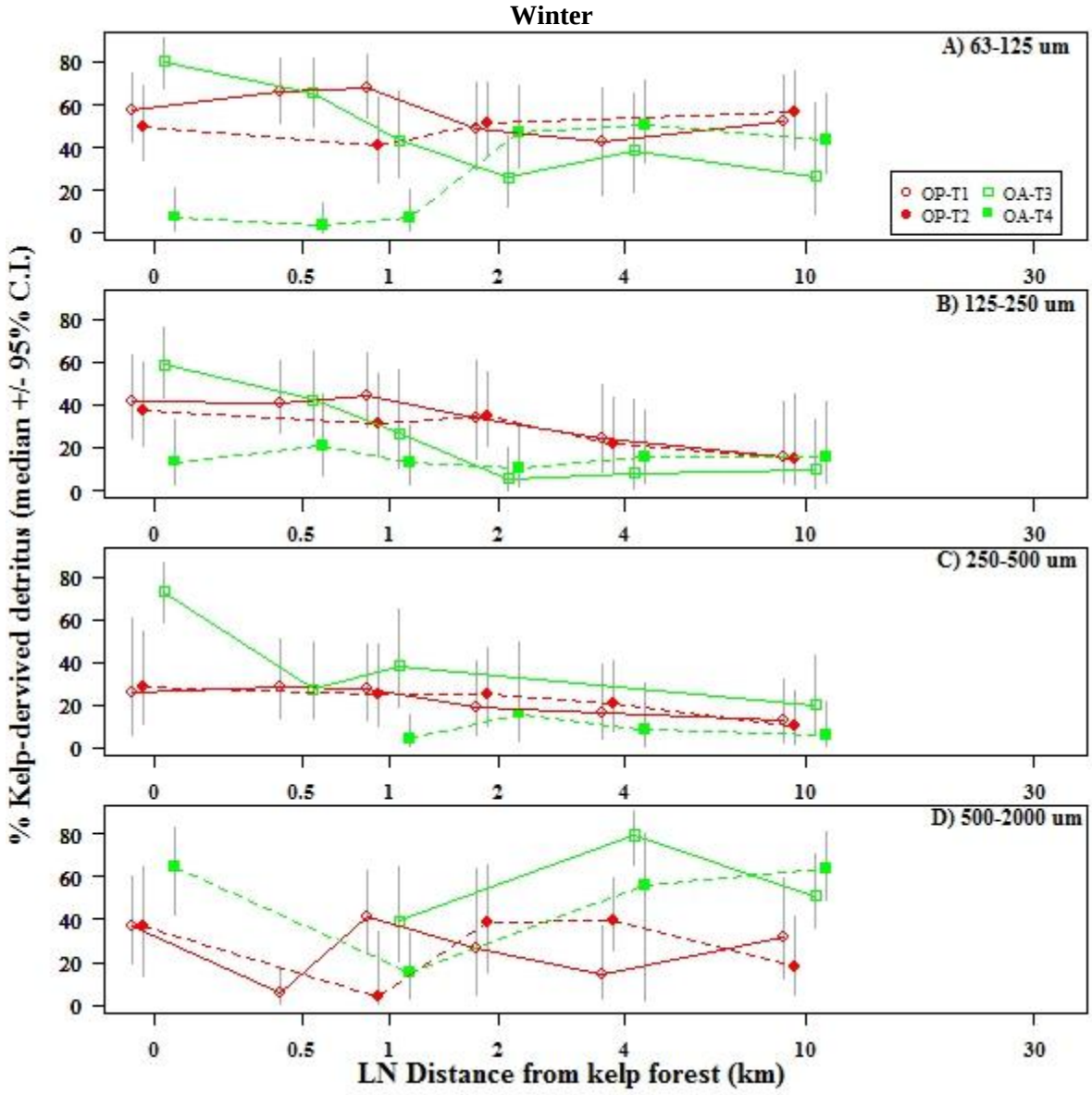

Figure 3.8. Winter percent kelp-derived detritus contribution (median ± 95 % C.I.) to surface 63 – 125 µm, 125 – 250 µm, 250 – 500 µm, and 500 – 2000 µm plankton for otter-preset and absent regions. Each point is a single sample (n = 1). For display purposes points are jittered horizontally.

sampling locations were composed of 0 to 5 % KDD (Fig. 3.9). The otter-intermediate region KDD contribution medians were from 64 to 71 % (52 to 85 % maximum confidence interval range) with both sampling sites being quite similar due to the large overlap in confidence intervals. The otter-absent region samples were composed of the largest percent KDD with median contributions from 68 to 98 % (Fig. 3.9). Seppings Island had the highest median percent KDD contribution with 97% (94 to 99 % C.I.) which was different from any other sampling site.

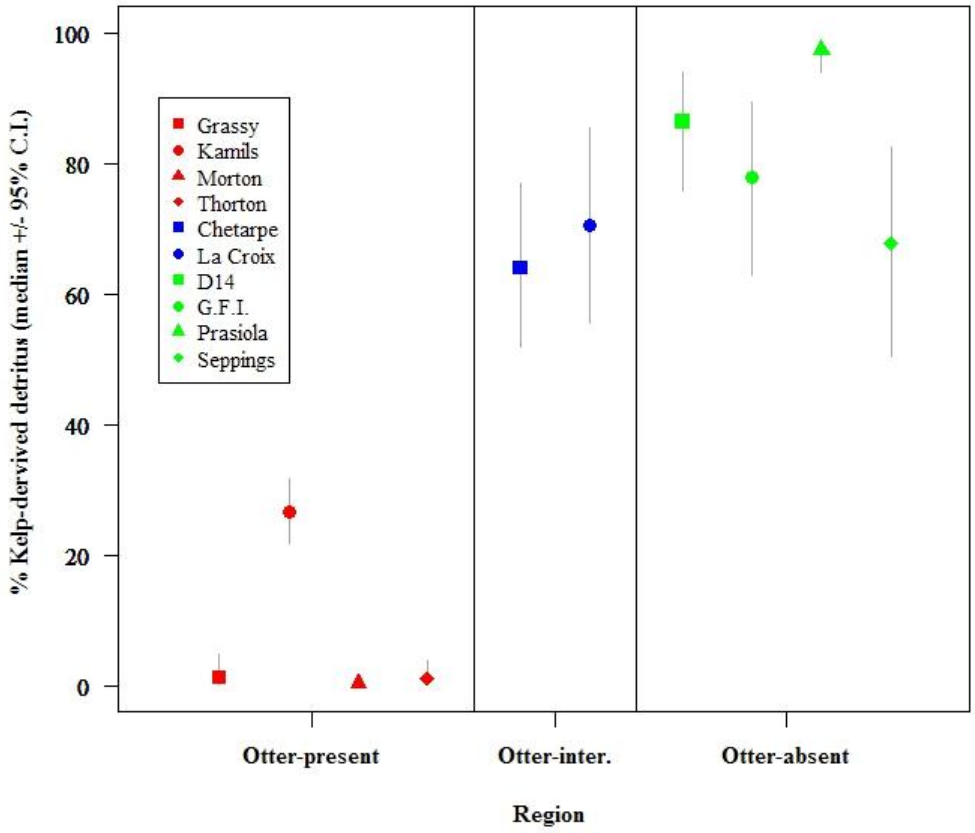

Figure 3.9. Percent kelp-derived detritus contribution (median ± 95 % C.I.) to *Astraea gibberosa* (red turban snail) during the summer. Within region sites are represented by different symbols. Samples sizes from left to right are Grassy: 1, Kamils: 7, Morton: 3, Thorton: 2, Chetarpe: 5, La Croix: 1, D14: 5, G.F.I.: 3, Prasiola: 5 and Seppings: 11.

Calliostoma: This genus of snails had decreasing $\delta^{13}C$ values with shell width ($R^2 = 18$, $p = 0.01$) so size classes were arbitrarily setup in half centimeter groupings to model in MixSIR. *Calliostoma* sp. were only collected in the dredge during the summer and inadequate spatial coverage with distance from the kelp forest prohibited regional and variation with distance comparisons (Fig. 3.10).

In the otter-present region, KDD contribution was high to all size classes with median contributions from 71 to 75 % and a maximum confidence interval range of 60 to 83 % with all size classes combined.

The 1.1 – 1.5 cm *Calliostoma* in the otter-absent region was composed of 92 % (85 to 93 % C.I.), which was a greater percentage of KDD than the same size class in the otter-present region and the 1.6 – 2 cm and 2.1 – 2.5 cm size class within this region (Fig.3.10).

Modeled estimates of KDD contribution within the otter-present and intermediate regions showed no decrease with size; however, the otter-absent region showed a trend of decreasing KDD contribution with increasing size.

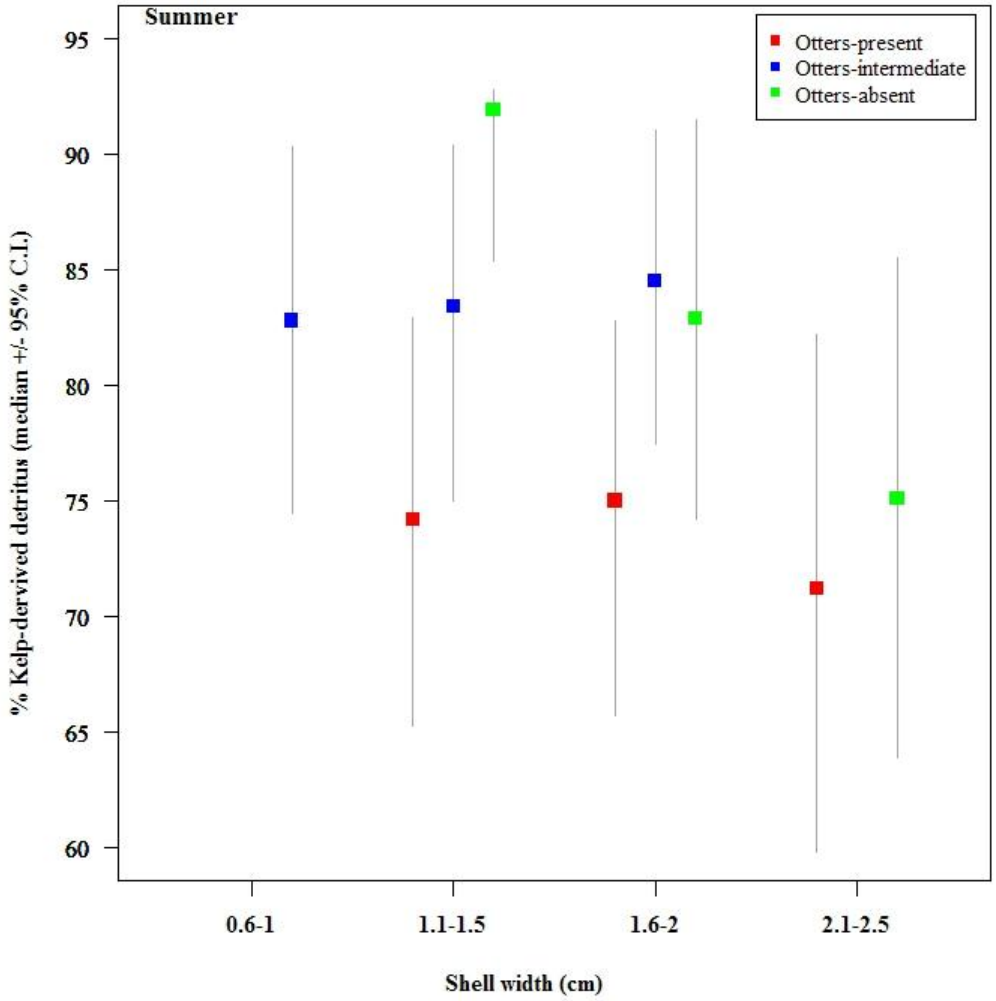

Figure 3.10. Percent kelp-derived detritus contribution (median ± 95 % C.I.) to *Calliostoma* sp. by shell width during the summer. Sample sizes from left to right are: 2, 5, 5, 4, 4, 8, 3, 1 and 2. Samples of the same shell width are jittered for display purposes.

Chlamys: When all three regions were combined, scallop summer $\delta^{13}C$ values increased with shell width ($R^2 = 0.43$, $p < 0.001$). This led to modeling KDD contribution to scallops grouped by shell width. Generally, during the summer percent KDD contribution to pink and spiny scallops was higher in the otter-absent region, especially in the larger size classes (Fig. 3.11). When all otter present-region size classes were combined, median percent KDD contributions were from 25 to 60 % (16 to 76 %, maximum possible contribution range) with the smallest size class (1.1 – 1.5 cm) having the largest median percent contribution of 60 % (47 to 72 % C.I.). Only one size class was collected from the otter-intermediate region during the summer. This size fraction (2.6 – 3 cm) had a percent KDD contribution of 62 % (48 to 76 % C.I.). Median percent KDD contributions to the otter-absent region scallops were from 75 to 92 % (64 to 93 %, maximum possible confidence interval). There was a trend towards increasing KDD contribution with size-class. Scallops of the smallest size class (1.1 – 1.5 cm) had a median percent contribution of 62 % (47 to 77 % C.I.) and the largest size class (3.6 – 4 cm) had a contribution of 81 % (72 to 89 % C.I.).

During the winter, scallops were only retrieved during dredging in the otter-present region. Percent KDD contribution to these scallops ranged from 40 to 73 % and there was no trend of increasing contribution with larger size classes (Fig 3.11). There was a trend of higher contribution during the winter relative to the summer in the otter-present region.

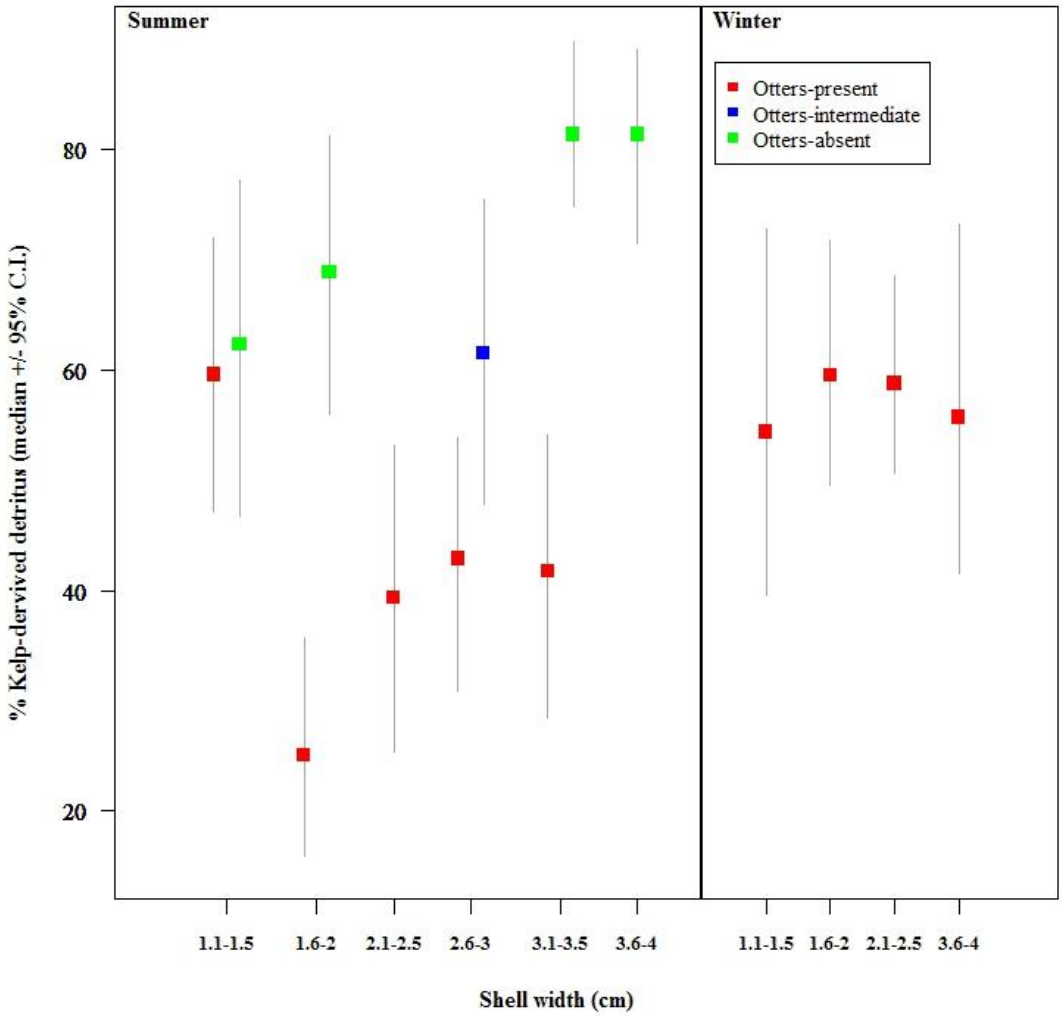

Figure 3.11. Percent kelp-derived detritus contribution (median ± 95 % C.I.) to *Chlamys* sp. (pink scallops) by shell width (cm). Winter samples were only retrieved from the otter-present region. Sample sizes from left to right are: 1, 1, 9, 1, 1, 3, 1, 2, 4, 3, 1, 6, 7 and 1. Samples of the same size class are jittered for display purposes.

Anomurans: Paired t-tests of hermit crab samples that were split in half and one half was treated with acid and the other half left untreated showed no change in $\delta^{13}C$ and $\delta^{15}N$ values. Further analyses were only performed on untreated samples.

Hermit crab $\delta^{13}C$ and $\delta^{15}N$ values increased with carapace width during the summer (R^2 = 0.40, $p < 0.001$ and $R^2 = 0.14$, $p < 0.001$, respectively) and during the winter with $\delta^{13}C$ (R^2 = 0.35, $p = 0.003$). Therefore, modeled KDD contribution estimates were determined for hermit crabs grouped in 0.1 cm bin intervals.

During the summer in the otter-present region, the two smallest size classes (0.2 and 0.3 cm) had the lowest contribution of KDD with the 0.2 cm size class having a median percent contribution 47 % (35 to 57 % C.I.). There was a trend towards increasing KDD contribution with increasing size (little confidence interval overlap) as the 0.8 cm size class having a possible median contribution of 76 % (66 to 85 % C.I.; Fig. 3.12). In the otter-intermediate region, median percent KDD contributions to hermit crabs were from 56 to 79 % (42 to 87 %, maximum possible contribution range). The 0.3 cm size class had the lowest contribution at 56 % (42 to 73 % C.I.), while the 0.4 cm size class had the largest at 79 % (68 to 87 %, C.I.). In the otter-absent region, the percent KDD contribution was high in all size classes. The median contributions were from 69 to 89 % (58 to 93 %, maximum possible contribution range) with 3 size classes having possible KDD contributions ≥ 90 % for 3 size classes (0.3, 0.4 and 0.6 cm). The otter-absent region 0.3 and 0.4 size classes had a larger percent KDD contribution than the similar otter-present region size classes.

During the winter, samples were only obtained in the otter-present and absent regions. Possible percent-kelp derived detritus contributions remained as high as during the summer. The median percent contribution for the 0.7 cm size class in the otter-present region was 73 % (60 to 86 %, maximum possible contribution range; Fig. 3.12) which was within the confidence intervals for most summer size-classes. In the otter-absent region the median percent KDD contributions were from 69 to 91 % (56 to 96 %, maximum possible contribution range) when all size classes were combined. The 0.2 cm size class had the lowest contribution 69 % (56 to 83 % C.I.) and the 0.5 cm size class had the highest contribution at 91 % (84 to 96 % C.I.); however, in general, there was high overlap in confidence intervals among most size-classes.

Fish: Sculpins (Family Cottidae) and flatfish (Family Pleuronectidae and Paralichthyidae) were only caught during the summer. Sculpin samples were grouped by length when percent KDD contributions were modeled as they had increasing $\delta^{13}C$ with length ($p = 0.03$, $R^2 = 0.57$). Flatfish samples were grouped by distance from the kelp forest as they had decreasing $\delta^{13}C$ with increasing distance ($p < 0.01$, $R^2 = 0.77$).

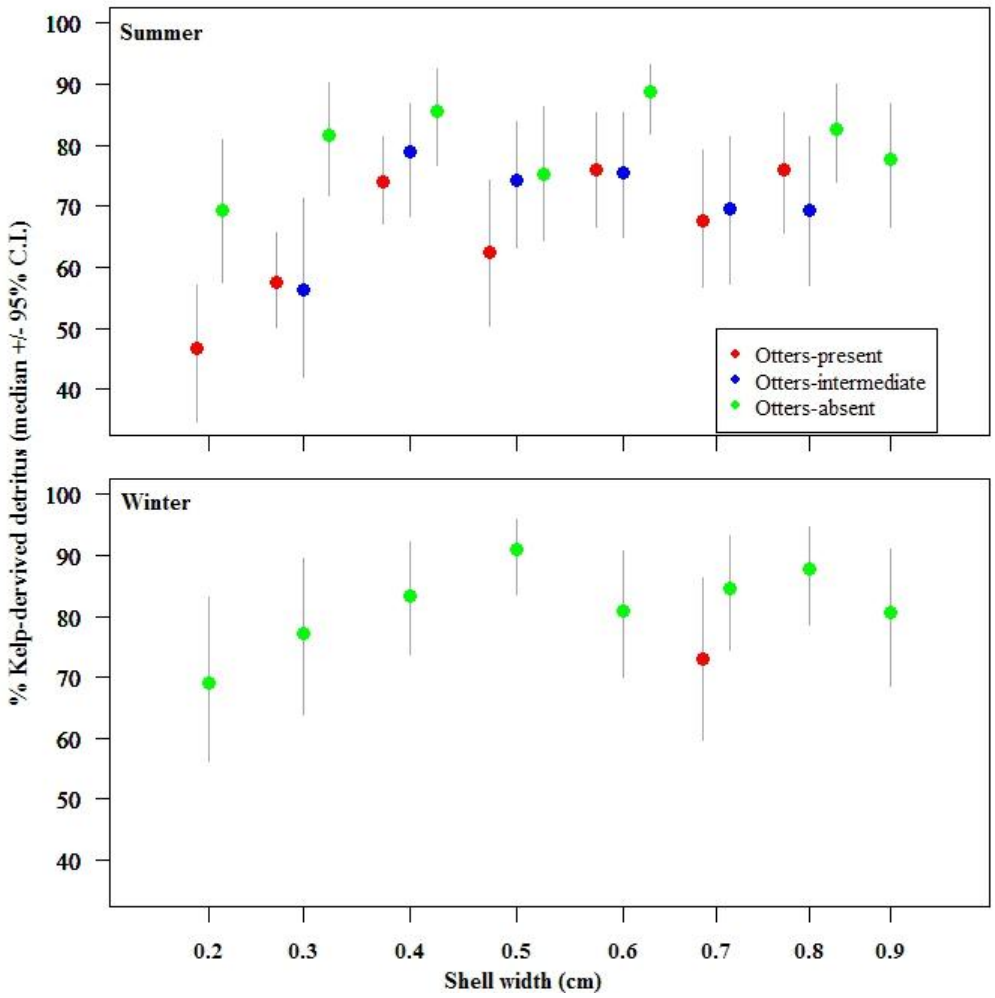

Figure 3.12. Percent kelp-derived detritus contribution (median ± 95 % C.I.) to hermits crabs by shell width (cm) for summer and winter along the otter-abundance gradient. Samples are pooled from a variety of distances from the kelp forests. Summer sample sizes from left to right; otter-present: 3, 5, 6, 1, 2, 1, 1; otter-intermediate: 1, 4, 5, 3, 2, 1; otter-absent: 2, 14, 2, 1, 4, 1, 1. Winter sample sizes from left to right; otter-present: 1; otter-absent: 2, 1, 5, 6, 3, 2, 1. Samples of the same width are jittered for display purposes.

The median percent KDD contributions to sculpins were from 16 to 74 % (2 to 86 %, maximum possible contribution range) with the lowest contribution being 16 % (2 to 38 % C.I.) for a 3.4 cm long individual from the otter-absent region. The largest contribution (74 %, 59 to 86 % C.I.) was for a 11.2 cm long individual also from this region (Fig. 3.13). There was no trend of increasing median KDD contribution to larger individuals and generally a high degree of overlap in confidence intervals among size-classes.

The median percent KDD contributions to flatfish were from 50 to 76 % (33 – 88 % maximum possible contribution range; Fig. 3.13) when all three regions were combined. There was a slight trend towards decreasing median percent KDD contributions with distance from the kelp forest; however, in general confidence intervals had relatively large overlap. There was only one sample from the otter-present region at 1 km from the kelp forest where the median KDD contribution was 50 (33 to 65 % C.I.). In the otter-intermediate region there was no difference with distance among samples with distance from the kelp forest. Median percent KDD contributions were from 58 to 65 (41 to 77 %, maximum possible contribution range) and there was large overlap among confidence intervals. The otter-absent region had similar median percent KDD contributions and large overlap in confidence intervals as well. Median percent KDD contributions were highest for the otter-absent region but the confidence intervals for these estimates had large overlap with the otter-intermediate but to a lesser degree with the otter-absent region.

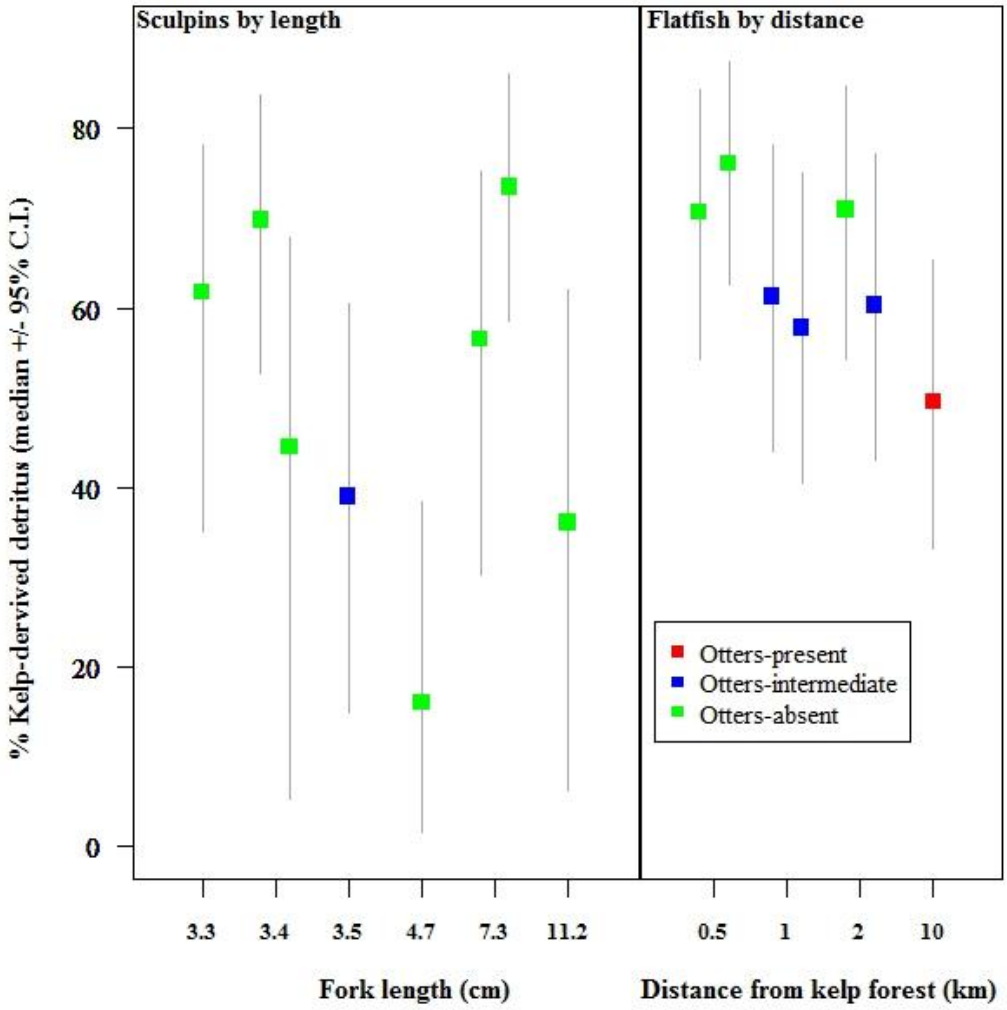

Figure 3.13. Percent kelp-derived detritus contribution (median ± 95% C.I.) to sculpins (Family Cottidae) by length and to flatfish (Families Bothidae and Pleuronectidae) with distance from the kelp forest (km) along the otter-abundance gradient. Sculpin samples are pooled from a variety of distances from the kelp forests. Sculpin sample sizes from left to right are: 1, 2, 1, 1, 2 and 1. Flatfish sample sizes are single individuals (n = 1). Samples are jittered for display purposes.

3.4 Discussion

The 'otter-urchin-kelp' trophic cascade has positive indirect effects on kelp abundance (Estes and Palmisano 1974) and previous studies have shown that kelp and KDD are an important food source for pelagic and benthic organisms (e.g. Dunton and Schell 1987, Duggins et al. 1989, Kaehler et al. 2000). In Alaska, Duggins et al. (1989) compared islands with sea otters and abundant kelp populations to islands without sea otters and dominated urchin barrens.

These authors found that primary consumers at islands with otters and abundant kelp were composed of nearly 60 % kelp-derived carbon compared to approximately 30 % at urchin dominated islands (Duggins et al. 1989). Similarly, kelp abundant population in South Africa are associated with organisms composed of up to 80 % kelp-derived carbon (Bustamante and Branch 1996). Based on these studies and others, we tested the hypothesis that kelp abundance being approximate 20-times greater in the presence of sea otters on the WCVI (Markel 2011) should be associated with benthic and pelagic organisms having greater dietary contributions of kelp derived detritus. Furthermore, during the winter, the senescing and dying kelp forests would be a stable, year-round food source as phytoplankton production is low (Bustamante and Branch 1996). It was further hypothesized that consumers in the presence of otters and kelp should have higher KDD contents during winter months as a result of correspondingly high abundance of senescing and decaying kelp tissues.

Contrary to expectation, regional differences in kelp contribution to primary consumers were not detected despite a 20 fold difference in kelp abundance between regions. Furthermore, no decrease in kelp contribution to primary consumers with increasing distance from source kelp forests within any region was found. As predicted, during the winter, KDD contribution to plankton was lower than during the summer and there was more variability with distance from the kelp forests and between regions. KDD contribution to benthic organisms was high in all regions during both summer and winter months except for *Astraea* in the otter-present region where KDD contribution was 41.2 to 96.6 % lower than the otter-absent region. Contrary to expectation, regional differences in kelp contribution to primary consumers were not detected despite a 20 fold difference in kelp abundance between regions. Furthermore, no decrease in kelp contribution to primary consumers with increasing distance from source kelp forests within any region was found. As predicted, during the winter, KDD contribution to plankton was lower than during the summer and there was more variability with distance from the kelp forests and between regions. KDD contribution to benthic organisms was high in all regions during both summer and winter months except for *Astraea* in the otter-present region where KDD contribution was 41.2 to 96.6 % lower than the otter-absent region.

3.4.1 Plankton $\delta^{13}C$ variability

During the summer and winter, few (30 % and 25 %, respectively) size fractions of plankton along a particular transect showed significant decreasing $\delta^{13}C$ values with distance from source kelp forests (Fig. 3.2). This was expected in the otter-present region as there is considerably more kelp production that is relatively ^{13}C enriched and may be transported offshore by ocean currents. The otter-intermediate region only had decreasing $\delta^{13}C$ values with distance from the kelp forest for the 250 – 500 µm fraction along both transects (Fig. 3.2, panel E and F). This may reflect the plankton composition of this fraction feeding on less kelp-derived production, or possibly the lack of kelp fragments within this larger fraction (Fig. 3.2 and 3.3). Additionally, larger plankton may be depleted in ^{13}C relative to smaller size fractions as lipids are ^{13}C depleted (McConnaughey and McRoy 1979) and if plankton accumulate large amounts

of lipids, this may shift their isotopic ratio (Kling et al. 1992). The vertical migration of plankton may play an important role as well if larger zooplankton or different species are migrating deeper where they are feeding on phytoplankton that have photoassimilated ^{13}C depleted CO_2 (Post 2002). Plankton $\delta^{13}C$ from the otter-absent region was more depleted with distance along transect 4 for all plankton smaller than 500 µm (Fig. 3.2). This is likely due to their food source (surface POM) being more depleted with distance along this transect as well (data not presented here). However, it is surprising that plankton from transect 3 remained relatively enriched in ^{13}C with distance from the kelp forest. This may result from the presence of KDD in these samples or the presence of blooming phytoplankton, which have been shown to have enriched $\delta^{13}C$ values (Fry and Wainright 1991; Chapter 1).

3.4.2 Plankton $\delta^{15}N$ variability

Plankton $\delta^{15}N$ variability is affected by the $\delta^{15}N$ variability of its food sources (Altabet and Francois 1994), isotope fractionation during assimilation (Schell et al. 1998) and excretion (Checkley and Miller 1989), and their trophic level (Minagawa and Wada 1984, Post 2002). A wide variety of animals are enriched in ^{15}N by ~1.5 – 4.5 ‰ relative to their diet and trophic position can be estimated from their $\delta^{15}N$ values (Post 2002, McCutchan et al. 2003). Although the causes of this enrichment are not completely understood, catabolic and excretory processes preferentially assimilate ^{15}N within animal tissues (Bada et al. 1989, Gannes et al. 1997).

KDD can support higher densities of consumers at higher trophic levels (Polis and Hurd 1996, Moore et al. 2004, Markel 2011). In this study, however only among region differences within plankton size fractions were found during the summer between the otter-present and absent region 500 – 2000 µm fraction with the otter-present region being 1.9 ‰ more enriched (data not presented here). However, this is likely not biologically significant, nor is it prevalent enough to conclude whether or not otter-present region plankton are feeding at higher trophic levels. There were no within-region differences among $\delta^{15}N$ values of plankton size fractions, which inhibits the conclusion that larger size fractions of plankton were feeding at higher trophic levels (Montoya et al. 2002).

3.4.3 Incorporation of kelp-derived detritus by plankton

KDD contribution to most plankton size fractions was moderate to high during the summer in all regions (Fig. 3.7). Contrary to our predictions, no regional differences in kelp contribution and no decrease in kelp contribution with distance from source kelp forests within any region were found. The two largest size fractions (250 – 500 and 500 – 2000 µm) had the most spatial variability. Uncertainties in the percent KDD estimates were greatest for the otter-absent region which could be misleading to how important KDD is to this region. This large uncertainty in the modeled estimates is likely the result of *Nereocystis* being the only kelp considered to be

contributing to POM in this region. This species had a mean $\delta^{13}C$ value of -18.31 ‰ which was closest to blooming phytoplankton values of -18.91 ‰ than in any other region.

During the winter there was greater spatial variability within regions and pronounced differences in detritus contribution between transects (Fig. 3.8). In general, KDD contribution was similar during the winter compared to summer; however, during the winter kelp contribution was patchier, especially in the otter-absent region.

It is important to determine accurate regional kelp species proportions as this may affect the mixing model output and lead to complications in interpreting the results or inaccurate conclusions. For example, in the otter-absent region, adjusting its kelp species proportions to include *Macrocystis* would enrich the $\delta^{13}C$ value used for kelp in the mixing model. This would decrease the KDD contribution to plankton within this region, likely decrease confidence intervals, and be more similar to what was predicted. It would be beneficial to future studies to incorporate a sampling design that captured regional species proportions as these can be highly variable (Berry et al. 2005, Watson and Estes 2011). A sensitivity analysis was conducted by pooling individual kelp species isotope values for the entire coast. This led to the depletion of 4 out of the 6 values (2 carbon and 2 nitrogen), and an increase in isotope value standard deviations in 5 out of the 6 cases. The mixing model returned similar median percent KDD values; however, the confidence intervals were larger in most cases due to the increased standard deviations, as a result of pooled regional values, inputted into the model.

3.4.4 Incorporation of kelp-derived detritus by benthic invertebrates

KDD incorporated by the snail *Astraea gibberosa* differed greatly within regions (Fig. 3.9). The stable isotope values *Astraea gibberosa* differed greatly among sites within the otter-present region, and therefore they could not be combined for mixing model estimates. Individuals from Kamils Island were most enriched (27 % KDD, 22 to 32 % C.I.), while those from the other sites were low (0 to 5 %). Surprisingly, the KDD contribution to the other two regions was high with a median contribution of 68 % (51 to 83 % C.I.) 0.5 km from the kelp forest (site D14) with a median contribution of 97 % (94 to 99 % C.I.) at Seppings Island. One explanation for these results may be that abundant kelp populations in the otter-present region is also associated with abundant understory red algae in the otter-present region (Martone and Markel, in prep). Red algae are highly depleted in ^{13}C (~ -30 ‰; Raven et al. 2002) and could be an abundant food source in the otter-present region and lead to the low contribution of kelp-derived production to these organisms, or as some have suggested, there is superiority in mixed diets compared to single foods (Westoby 1978, Pennings et al. 1993, Bernays et al. 1994). A second explanation for the individuals in the otter-present region being more ^{13}C depleted is that on average they are approximately 2 cm smaller. Sea otter populations at equilibrium density are thought to be food limited (Kenyon 1969) and changes in prey availability will influence sea otter foraging behaviour (Estes et al. 1982). Sea otters preferentially feed on sea urchins when available (Estes

and Palmisano 1974, Estes et al. 1982); however, in areas where otters have been established for quite some time large urchins are absent (Estes and Palmisano 1974) and otters consume less preferred prey that have become more energetically profitable (Ostfeld 1982, Tinker et al. 2008). This is likely what has happened in the otter-present region and large *Astraea* are heavily preyed upon and reduced in abundance. Smaller *Astraea* may eat less kelp tissue and consume diatoms and bacteria on kelp blades that are relative ^{13}C depleted (DeNiro and Epstein 1978, Velji and Albright 1986, Duggins et al. 1989), or be structurally limited due to smaller or weaker mouth parts and not be able to consume, or possibly even digest kelp, due to its rigidness (Padilla 1985).

In general, modeled estimates of KDD contribution to benthic invertebrates and fish were similar with size, among regions and between seasons. The incorporation of KDD by *Calliostoma* was similar and high (> 60 %) for all regions (Fig. 3.10). This was not the case for *Chlamys* spp. as otter-absent region scallops had higher contributions of KDD than the otter-present region for most size classes (Fig. 3.11). The otter-intermediate region was midway between the other two regions. During the winter there was a trend towards a higher contribution of KDD for all size classes of scallop in the otter-present region. Hermit crabs showed a similar pattern except for the two smallest size classes (0.2 and 0.3 cm) in the otter-present region were composed of less KDD than the otter-absent region (Fig. 3.12). This is evidence of possible hermit crab body size effects with respect to KDD consumption. Again, during the winter the contribution remained as high as during the summer, which was consistent across all size classes. Lastly, all size classes of sculpin had similar KDD contribution estimates across regions, as did contributions to flatfish regardless of their distance from the kelp forests (Fig. 3.13).

The results of this study indicate that the moderate to high KDD contribution to plankton, most benthic invertebrates and fish year-round at all distances offshore of the otter-present region is likely due to the oceanographic conditions specific to this region, that alter the abundance of KDD. Although these contribution estimates are potentially high, it was predicted that the KDD contribution would be consistently higher. Summer growth and accumulation of kelp biomass is released into the environment through senescence and dissolved organic carbon (Lucas et al. 1981). Winter storms accelerate the breakdown of kelp and release the biomass gained in the summer during the winter (Dayton 1985). Harris (2001) postulated that the water beyond the continental shelf off northern Vancouver Island was similar to shelf water due to the narrow (~5 km) continental shelf along the northern part of Vancouver Island. Wind driven upwelling filaments regularly form off Brooks Peninsula (just north of the otter-present region; Fig. 3.1) and move a significant proportion of the nutrients, phytoplankton and plankton off the shelf into the pelagic environment (Forbes and Denman 1991). Moreover, when the north-flowing VICC meets the Brooks Peninsula it is deflected offshore, occasionally forming eddies (Thomson et al. 1989), and may possibly draw the nearshore KDD away from shore. This study collected total POM near the ocean floor in the otter-present region (data not presented here) which was depleted in ^{13}C 1.6 ‰ relative to surface POM during the summer and 1.1 ‰ during the winter. Harris (2001) explained the SST gradient along the WCVI, with lower temperatures

off the otter-present region, as a result of the narrow shelf because upwelled water off the otter-present region has a shorter distance to travel and less time to warm before being transported off the shelf. This could have implications for water residence time over the shelf and upwelled water and particles with depleted ^{13}C being in the nearshore area depleting surface POM $\delta^{13}C$. Although KDD production may be relatively high in this region it is possible that the retention of this production in this region is reduced by regional oceanography and/or diluted by upwelled, ^{13} depleted POM.

The surprisingly high contribution of KDD to consumers from the otter-absent region during the summer and winter may be explained as follows. First, although kelp abundance is 20-times higher in the otter-present region, there is still plenty of kelp present in the otter-absent region (Martone and Markel, in prep) to contribute to this region's POM. Second, the $\delta^{13}C$ values of *Nereocystis* in this region being similar to the values of blooming phytoplankton resulted in modeled KDD contribution estimates with high uncertainty (i.e., large confidence intervals). Altering the kelp proportions in this region from 100 % *Nereocystis* to include *Macrocystis* would enrich the kelp $\delta^{13}C$ value used in the mixing model and potentially decrease the maximum possible contribution of KDD for organisms within this region. Estimate uncertainty was higher in both regions during the winter because kelp values used in the mixing model had larger standard deviations as a result of greater within region variation. The presence or absence of sea otters indirectly alters kelp forest species composition through successional processes and grazer-kelp interactions, respectively (Duggins 1980, Watson and Estes 2011), and therefore, may affect consumer diets and isotope values. Interestingly, drift kelp and KDD are known to accumulate on gentle slopes and depressions, and in areas of low current velocity (Britton-Simmons et al. 2012). Britton-Simmons et al. (2012) data suggests that drift material accumulates on low-angle habitats; however, localized hydrodynamics also play a role in its distribution. Biber (2007) reports that areas with low standing biomass of drift macroalgae and high currents can still have significant fluxes of drift pass through. These processes can lead to shifts in consumer spatial distributions in response to food to availability (Britton-Simmons et al. 2012) leading to isotope enrichment and potentially obscuring predicted patterns.

3.4.5 Summary

This study used region-specific kelp isotope values ($\delta^{13}C$ and $\delta^{15}N$) and season-specific phytoplankton isotope values to model estimates of KDD contribution to plankton and benthic organisms. In general, KDD contribution to most plankton size fractions was moderate to high during the summer but decreased during the winter (to a greater extent in the otter-absent region). Hypothesized regional differences in kelp contribution did not exist and in general there was no decrease in contribution with distance from the kelp forest within any region. Modeled estimates of KDD contribution to benthic invertebrates and fish were high (> 40 %) and similar with size, among regions and between seasons, with the exception of *Astraea gibberosa* in the otter-present region. The likely cause of this exception is the otter-present region *Astraea* were 2

cm smaller due to sea otter predation on larger individuals and smaller individuals may graze on diatoms or bacteria or be limited in their ability to consume macroalgae (Estes and Palmisano 1974, Ostfeld 1982, Padilla 1985, Tinker et al. 2008). Local oceanography, kelp forest species composition, kelp species isotope variation, and kelp's similarity with blooming phytoplankton isotope values led to unpredicted results and uncertainty in the modeled KDD contributions. Ultimately, this speaks to how widespread the indirect effects of otters (e.g., kelp forest community succession), and other sources of ecosystem change, can or may be.

Chapter 4: General Discussion

Sea otters move into new regions and feed heavily on sea urchins and initiate a top-down trophic cascade that allows the proliferation of kelp populations (Estes and Palmisano 1974, Estes et al. 1982). Kelp is highly productive with estimates ranging from 460 to 1300 g C m^{-2} yr^{-1} (Mann 1973, Coon 1982, Druehl and Wheeler 1986). Kelp blades senesce and breakdown during storms (Bustamante and Branch 1996) and forms kelp-derived detritus (KDD) which is an important autochthonous and allochthonous subsidy to nearshore, pelagic and benthic ecosystems (Bustamante and Branch 1996, Harrold et al. 1998, Kaehler et al. 2000, Jack and Wing 2011).

The reintroduction of sea otters to the west coast of Vancouver Island (WCVI) drastically changed nearshore ecosystems in many ways (Watson 1993, Nichol et al. 2005). Sea otters indirectly led to the increase of kelp abundance along much of its range (Watson and Estes 2011). In this study, it was hypothesized that this increase in kelp abundance would lead to marked differences in KDD in the water column surrounding an otter and kelp abundant region relative to an otter absent and low kelp abundance region. It was also predicted that the KDD would be found further offshore during summer and winter within the otter-present region and plankton and benthic invertebrates within this region would be composed of KDD to a greater extent.

To quantify the abundance of KDD in suspended particulate organic matter (POM) and its contribution to organisms it was necessary to determine regional and seasonal kelp and phytoplankton stable isotope values (e.g., Duggins et al. 1989) as they have been shown to vary over these scales (Rau et al. 1989, Foley and Koch 2010). For this study it was assumed that *Macrocystis*, *Nereocystis* and *Pterygophora* were the only three species contributing to POM along the WCVI. A concurrent study by Martone and Markel (in prep) determined that kelp communities differed among regions, and therefore, region-specific kelp proportions were used in the stable isotope mixing model. It was determined that there were no within species differences among regions with respect to $\delta^{13}C$, but the $\delta^{15}N$ value of *Nereocystis* differed between the otter-present and intermediate regions. There were a number of within region differences among species with respect to isotopes. In general, *Macrocystis* (summer $\delta^{13}C$: -13.17 to -14.42 ‰, and $\delta^{15}N$: 8.92 to 8.99 ‰) was the most enriched and *Pterygophora* (summer $\delta^{13}C$: -17.42 to -19.49 ‰, and $\delta^{15}N$: 6.82 to 7.59 ‰) was the most depleted in the case of both isotopes.

There has been a considerable amount of research on the factors that affect phytoplankton isotope variability. Studies have shown blooming phytoplankton to have enriched $\delta^{13}C$ values (Fry & Wainwright 1991), and some postulate that this is the result of the differential use of enzymes during photosynthesis and carboxylation (Guy et al 1989) when nitrogen uptake is elevated during periods of high growth rates (Guy et al 1989, Descolas-Gros and Fontugne

1990). Phytoplankton blooms in their early stages have depleted $\delta^{15}N$ values because phytoplankton utilize $N^{14}O_3^-$ first and then uses $N^{15}O_3^-$ as nitrate becomes limiting (Altabet and Francois 1994). Under nutrient limited conditions, such as the nearing the end of a phytoplankton bloom, all nitrate could be incorporated in its entirety by phytoplankton and have a $\delta^{15}N$ value similar to the source nitrate (Altabet and Francois 1994). In this study, the sampling of size-fractionated phytoplankton and oceanographic variables, and knowledge from past studies enabled the determination of size-specific isotope values and to distinguish between blooming and non-blooming phytoplankton isotope values and how these varied seasonally.

Phytoplankton blooms were only occurring during the summer and the areas experiencing blooms had enriched ^{13}C relative to non-blooming areas (~ -19 ‰ and ~ -24 ‰, respectively). During the winter, no phytoplankton blooms were occurring and the $\delta^{13}C$ values was similar to non-blooming summer value. In general, larger size fractions of phytoplankton were more enriched. For example, the > 0.7 µm phytoplankton (-18.91 ‰) was more enriched than the 0.7 – 20 µm fraction (-21.15 ‰).

The results of this study indicate that the moderate to high KDD contribution to total and 20 – 63 µm POM year-round at all distances offshore of the otter-present region is likely due to the oceanographic conditions specific to this region that reduce the abundance of KDD. KDD contribution to POM off of the otter-absent region was surprisingly high. During the summer, the nearshore contribution of KDD to POM rivaled or exceeded that of the otter-present and intermediate regions. During the winter, the contribution to POM decreased in the otter-absent region but remained high at a few distances.

The contribution of KDD to plankton was similar spatially and temporally to its contribution to POM. KDD contribution to most plankton size fractions was moderate to high during the summer in all regions. Contrary to our predictions, no regional differences were found in kelp contribution and no decrease in kelp contribution with distance from source kelp forests within any region. The two largest size fractions (250 – 500 and 500 – 2000 µm) had the most spatial variability. Similar to the POM estimates, uncertainties in the percent KDD contribution estimates to plankton were greatest for the otter-absent region which could be misleading to how important KDD is to this region. This large uncertainty in the modeled estimates is likely the result of *Nereocystis* being the only kelp considered to be contributing to POM in this region. This species had a mean $\delta^{13}C$ value of -18.31 ‰ which was closest to blooming phytoplankton values of -18.91 ‰ than in any other region. During the winter there was greater spatial variability within regions and pronounced differences in detritus contribution between transects. In general, KDD contribution was similar during the winter compared to summer; however, during the winter kelp contribution was patchier, especially in the otter-absent region.

There are potentially three possible explanations for these results. First, the role that bacteria play in the incorporation of kelp dissolved organic carbon (DOC) and degradation of KDD may impact POM isotope values. Bacteria cover the surface and actively degrade kelp, as

well as incorporate kelp-derived DOC to support their own productivity (Linley et al. 1981, Linley and Newell 1984). Fresh kelp may be unsuitable for certain grazers and may only become suitable after bacterial degradation of kelp blades and particles (Norderhaug et al. 2003). Bacterial degradation lowers C:N ratios and secondary metabolites of KDD, making it a more nutritional food source (Duggins and Eckman 1997, Pennings et al. 2000). Goldman et al. (1987) showed that bacteria growing at optimal conditions can reach C:N ratios as low as 4:1, and their high abundance and turnover rates (Clasen et al., unpubl. data) may lead to decreased ratios measured in the water column. Clasen et al. (unpubl. data) found that bacterial abundance and growth rates were significantly higher in the otter-present relative to the otter-absent region. Alginate lyase activity, a bacteria ectoenzyme involved in the degradation of kelp, is significantly higher in the otter-present compared to the otter-absent region, which could signify higher turnover rates and productivity in this region. The higher abundance of bacteria in the otter-present region may alter $\delta^{13}C$, $\delta^{15}N$ and C:N values of POM, thereby influencing the interpretation of these values and altering the relative contribution of KDD to POM and underestimating the contribution of KDD because bacteria has been shown to have a $\delta^{13}C$ of -24.4 ‰ (Deniro and Epstein 1978).

Second, just north of the otter-present region the north-flowing VICC meets Brooks Peninsula and is deflected offshore, occasionally forming eddies (Thomson et al. 1989), and may possibly pull the nearshore KDD away from shore. Harris (2001) explained the SST gradient along the WCVI from their data, with lower temperatures off the otter-present region, as a result of the narrow shelf because upwelled water off the otter-present region has a shorter distance to travel and less time to warm before being transported off the shelf. This could have implications for water residence time over the shelf and upwelled water depleted in ^{13}C being in the nearshore area depleting POM $\delta^{13}C$ (Post 2002, Raven et al. 2002). Polis and Hurd (1996) discuss the effect of ecosystem perimeter to area ratios and how the connectivity and flux will be greater in regions with larger ratios. This could apply to the regions in this study. The otter-present region has a narrower continental shelf, but a similar distance of coastline was studied among regions, therefore, the otter-present region area is smaller (shelf length x coastline length) and could be more easily affected by the input of pelagic and benthic POM sources that are generally more depleted. This coupled with local oceanographic processes mentioned above, lends the region depleted POM ^{13}C input or removal of ^{13}C enriched KDD. Although KDD production may be relatively high in this region it is possible that the retention of this production in this region is reduced by regional oceanography.

Third, regional differences with respect to the dominant kelp species influence the isotope values of POM, the diets of consumers, and their isotope values. Sea otters can initiate these regional differences because when they establish in a new region they consume grazers, such as sea urchins, and cause successional changes in kelp community composition (Duggins 1980, Watson and Estes 2011). For example, *Nereocystis* was markedly more abundant than *Macrocystis* in the otter-absent region (Martone and Markel, in prep). *Nereocystis* $\delta^{13}C$ values

were more depleted than *Macrocystis* during the summer and winter in both the otter-present and otter-absent regions. The higher abundance of *Nereocystis* in the otter-absent region, and its higher productivity and turnover rates, may result in this species making larger contributions of KDD to POM, plankton and benthic invertebrates, in general. Additionally, *Nereocystis* had a similar $\delta^{13}C$ value to blooming phytoplankton which caused uncertainty in modeled KDD contribution estimates in the otter-absent region.

The weaknesses of this study are the small sample size, relatively poor spatial coverage, pseudo-replication, and the short temporal scale. The sampling design of this study only allowed for the collection of a single measurement of each parameter which limits the ability of this study to make highly confident statements about within sample site variability. Obviously, due to limited time and money this is difficult to overcome and the designed allowed for as much spatial coverage as realistically possible. However, there were instances where multiple POM and plankton samples and multiple individuals of a single benthic invertebrate species were collected from the same site. These samples showed little variation and even though this is considered pseudo-replication it gives confidence to the measured isotope values. With only 6 transects to represent the entire WCVI it is likely that the sampling does not fully capture the potentially high spatial variability in isotope values. The sampling design of this study nests two transects within the same region and presents them as discrete measurements. Again, this is not an ideal situation but with the constraints of the study system (an otter abundance gradient) it is a best case scenario. Finally, with the dataset only containing measurements from one summer and winter, long term variability in space in time is not captured. These estimates could vary monthly or annually in relation to variation in kelp and phytoplankton productivity, ocean currents, and upwelling intensity.

The strengths of this study lie in its somewhat unique approach. Spatial and temporal variability in kelp isotope values were characterized, in addition to among species differences. This was paired with unique isotope values for size-fractionated phytoplankton into a Bayesian isotope mixing model (MixSIR) that incorporates uncertainty in kelp and phytoplankton isotope estimates to determine percent contribution of these two food sources. This approach is not common in the literature, and many studies use linear two-source mixing models that provide no uncertainty in percent KDD estimates. Additionally, regional kelp forest community composition was taken into consideration when determining region-specific isotope values to use for modeling KDD contribution estimates. Furthermore, the multi-study approach used to reveal these ecosystem processes in a complex study system allowed the multiple objectives of the study to collaborate, share data, and explain the connectivity of the system at multiple scales.

This study's results can be utilized by managers to advise decision making surrounding the reestablishment of sea otters and the importance of kelp, in general. When otters establish themselves in new areas they cause the proliferation of large kelp forests (Estes and Palmisano 1974) which are known to have many benefits with their physical structure, biomass, and their associated organisms (Steneck et al 2003). They provide 3-dimensional structural habitat

(Steneck et al. 2003 and references within), reduce local current velocities and dampen waves (Gaylord et al. 2007), and their canopies reduce irradiance at depth thereby affecting understorey conditions and species assemblages (Santelices and Ojeda 1984). Kelp is eaten directly by organisms (Bustamante and Branch 1995) and provides foraging habitat for kelp associated fishes (Reisewitz et al 2006, Norderhaug and Christie 2010). These benefits need to be taken into consideration when analyzing the ecosystem level changes that occur when otters re-establish and used as part of any ecosystem based management approach. Finally, the creation of habitat for recreationally and commercially important fishes is important for economic, conservation and species diversity initiatives.

Future research in modeling the relative importance of KDD to marine ecosystems needs well designed studies that can account for spatial and temporal variation in stable isotope values. This concept is fundamental in characterizing the spatial and temporal variability that may exist in KDD contribution to food webs. Future studies should increase replication of all POM samples collected and correspond with a comprehensive suite of oceanographic variable measurements, including $[CO_{2(aq)}]$. This study used regional kelp species proportions based on abundance but an interesting exercise would be to include all kelp species in the mixing model separately and obtain species specific contributions within each region. It would be beneficial to also separate plankton by species and obtain phytoplankton and zooplankton isotope values for specific species or groups. Studies that can identify all possible food sources, measure their isotope values, capture their variability and recognize factors responsible for isotopic variation will be well suited to modeling estimates with high confidence.

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Appendix A: Chapter 2 Supplementary Figures and Tables

	Otter-present			Otter-intermediate			Otter-absent		
Summer	Mean (µg/L)	S.D. (±)	Range (µg/L)	Mean (µg/L)	S.D. (±)	Range (µg/L)	Mean (µg/L)	S.D. (±)	Range (µg/L)
Total (>0.7 µm)	7.16	2.54	1.66 - 10.71	5.48	1.98	3.14 - 9.29	8.80	4.35	3.01- 14.35
Nearshore (≤2 km)	8.06	0.64	6.87 - 8.74	6.24	2.17	3.84 - 9.29	7.89	3.94	3.01 - 14.09
Offshore (≥4 km)	6.25	3.45	1.66 - 10.71	4.56	1.21	3.14 - 6.66	10.0	4.94	3.45 - 14.35
>20 µm	6.4	2.73	0.92 - 10.25	5.89	2.1	3.06 - 9.05	7.96	4.57	2.45 - 15.13
Nearshore (≤2 km)	7.22	1.58	5.88 - 10.25	7.0	1.52	4.76 - 8.89	7.02	3.88	2.48 - 13.51
Offshore (≥4 km)	5.57	3.51	0.92 - 9.71	4.07	0.97	3.06 - 5.75	9.21	5.47	2.45 - 15.13
Winter									
Total (>0.7 µm)	0.25	0.06	0.16 - 0.53	0.38	0.1	0.26- 0.49	0.30	0.19	0.05 - 0.55
Nearshore (≤2 km)	0.31	0.1	0.16 - 0.44	n/a	n/a	n/a	0.30	0.19	0.05 - 0.55
Offshore (≥4 km)	0.34	0.1	0.22 - 0.53	0.38	0.1	0.26- 0.49	0.33	0.05	0.25 - 0.37
>20 µm	0.21	0.05	0.14 - 0.29	0.25	0.05	0.19- 0.28	0.20	0.11	0.04 - 0.40
Nearshore (≤2 km)	0.20	0.04	0.14 - 0.25	n/a	n/a	n/a	0.19	0.13	0.04 - 0.40
Offshore (≥4 km)	0.23	0.07	0.14 - 0.29	0.25	0.05	0.19- 0.28	0.23	0.04	0.18 - 0.26

Table A1. Mean total (> 0.7 µm) and > 20 µm chl *a* concentration (µg/L) ± SD and ranges during summer and winter among all regions. 'Nearshore' is defined as ≤ 2 km from shore and 'offshore' is defined as ≥ 4 km from shore.

	Otter-present			Otter-intermediate			Otter-absent		
Summer	**Mean (µmol/L)**	**S.D. (±)**	**Range (µmol/L)**	**Mean (µmol/L)**	**S.D. (±)**	**Range (µmol/L)**	**Mean (µmol/L)**	**S.D. (±)**	**Range (µmol/L)**
Overall	1.69	1.13	0.05 to 4.04	3.54	3.76	0 to 9.53	0.04	0.10	0 to 0.27
Nearshore (≤2 km)	2.19	1.11	1.12 to 4.04	5.54	3.57	0 to 9.53	0	0	0 to 0
Offshore (≥4 km)	1.20	0.98	0.05 to 2.99	0.88	2.01	0 to 4.99	0.09	0.15	0 to 0.27
Winter									
Overall	10.97	0.50	9.55 to 11.43	12.33	1.42	11.08 to 13.87	10.76	1.30	8.85 to 13.68
Nearshore (≤2 km)	11.20	0.15	11.0 to 11.44	nd	nd	nd	10.49	1.15	8.85 to 12.01
Offshore (≥4 km)	10.52	0.67	9.55 to 11.07	12.33	1.42	11.08 to 13.87	11.30	1.60	10.34 to 13.68

Table A2. Regional summer and winter mean nitrate concentration (µmol/L ± SD) and range. The mean nearshore (≤ 2 km) and offshore (≥ 4 km) nitate concentration (µmol/L ± SD) and range within each region.

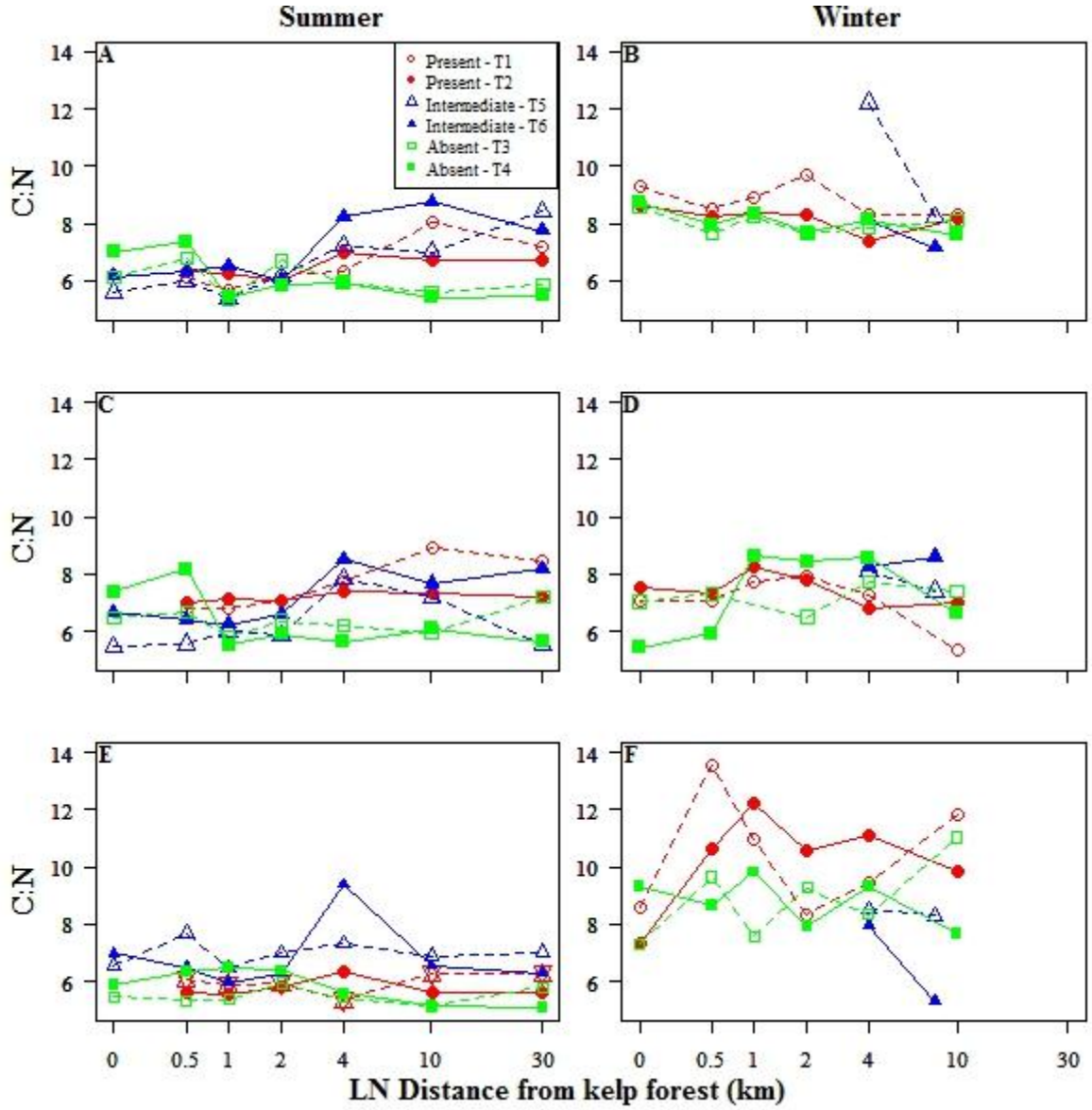

Figure A1. Summer (left column) and winter (right column) $\delta^{13}C$ values (‰) for total POM (> 0.7 µm; panel A and B), 20 – 63 µm POM (panel C and D) and 0.7 – 20 µm POM (panel E and F) with natural log distance from the kelp forest for transects from all regions. Each point represents a single sample (n=1).

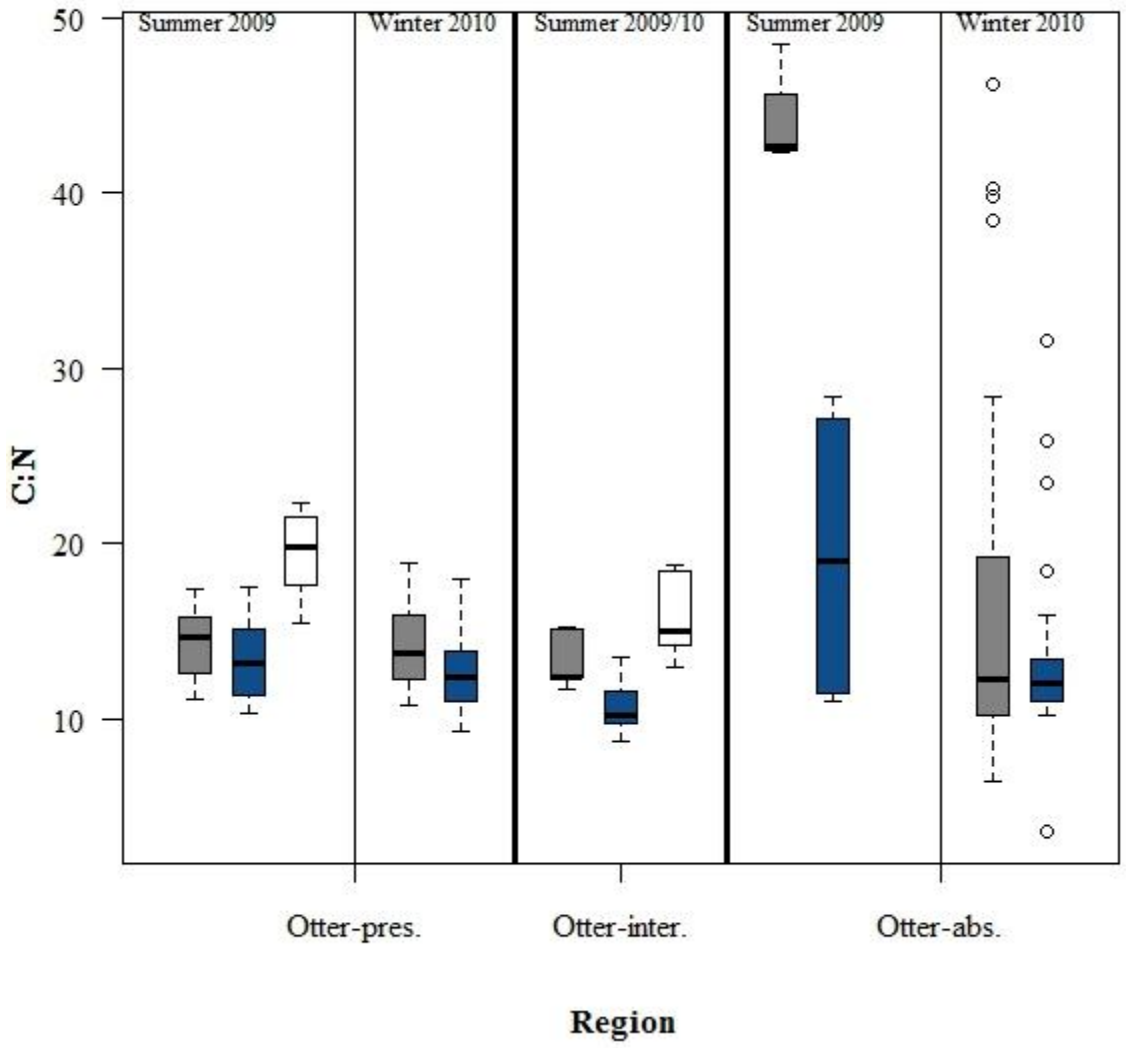

Figure A2. Regional summer and winter *Macrocystis*, *Nereocystis* and *Pterygophora* C:N (unit/unit). Grey bars represent *Macrocystis*, blue bars represent *Nereocystis*, and white bars represent *Pterygophora*. No data available for *Pterygophora* during winter in all regions and for summer in the otter-absent region. Medians are represented by black horizontal lines.

Summer	Otter-present			Otter-intermediate			Otter-absent		
Species	**Mean C:N**	**SD (±)**	**Range**	**Mean C:N**	**SD (±)**	**Range**	**Mean C:N**	**SD (±)**	**Range**
Macrocystis pyrifera	14.29	2.00	11.15 to 17.41	13.35	1.64	11.72 to 15.19	44.48	3.51	42.27 to 48.52
Nereocystis luetkeana	13.42	2.35	10.32 to 17.53	10.74	1.70	8.74 to 13.50	19.32	9.09	11.02 to 28.32
Pterygophora californica	19.51	2.19	15.39 to 22.24	15.81	2.61	12.88 to 18.74	nd	nd	nd

Table A3. Regional summer kelp mean C:N (unit/unit ± SD) and range for *Macrocystis*, *Nereocystis* and *Pterygophora*. No data ("nd") available for the otter-absent region *Pterygophora*.

Winter	Otter-present			Otter-intermediate			Otter-absent		
Species	**Mean C:N**	**SD (±)**	**Range**	**Mean C:N**	**SD (±)**	**Range**	**Mean C:N**	**SD (±)**	**Range**
Macrocystis pyrifera	14.02	2.22	10.76 - 18.82	nd	nd	nd	17.63	11.64	6.46 to 46.22
Nereocystis luetkeana	12.78	2.35	9.31 - 17.93	nd	nd	nd	13.53	5.55	3.55 to 31.58

Table A4. Regional winter kelp mean C:N (unit/unit ± SD) and range for *Macrocystis*, *Nereocystis*. No data ("nd") available for the otter-intermediate region.

Appendix B: Chapter 3 Supplementary Figures and Tables

	Kyuquot Sound *Macrocystis* **– 66.7%,** *Nereocystis* **– 33.3%**		**Clayoquot Sound** *Nereocystis* **– 80%,** *Pterygophora* **– 20%**		**Barkley Sound** *Nereocystis* **– 100%**	
Season	**Mean $\delta^{13}C$ (±) SD ‰**	**Mean $\delta^{15}N$ (±) SD ‰**	**Mean $\delta^{13}C$ (±) SD ‰**	**Mean $\delta^{15}N$ (±) SD ‰**	**Mean $\delta^{13}C$ (±) SD ‰**	**Mean $\delta^{15}N$ (±) SD ‰**
Summer	-14.55 ± 1.37	8.87 ± 0.93	-16.68 ± 1.70	7.19 ± 1.50	-18.31 ± 1.17	8.28 ± 1.13
Winter	-15.68 ± 2.29	7.69 ± 2.08	-15.58 ± 2.30	5.77 ± 1.66	-16.15 ± 2.27	6.80 ± 1.75

Table B1. Summer and winter kelp carbon and nitrogen stable isotope values (‰) ± SD used in MixSIR based on the regionally abundant kelp species (Martone and Markel, unpublished data).

		Summer		Winter	
POM size fraction	**Phytoplankton type**	**Mean $\delta^{13}C$ (±) SD ‰**	**Mean $\delta^{15}N$ (±) SD ‰**	**Mean $\delta^{13}C$ (±) SD ‰**	**Mean $\delta^{15}N$ (±) SD ‰**
Total POM	Blooming phytoplankton >0.7 µm	-18.91 ± 0.30	6.9 ± 0.63	No bloom	No bloom
	Non-blooming phytoplankton >0.7 µm	-24.23 ± 0.48	3.74 ± 0.80	-23.5 ± 0.70	7.81 ± 1.03
	Blooming 0.7 – 20 µm phytoplantkon	-21.15 ± 0.31	6.71 ± 0.93	No bloom	No bloom
	Non-blooming 0.7 – 20 µm phytoplantkon	-23.63 ± 0.28	6.37 ± 0.91	-24.42 ± 0.58	5.9 ± 1.06
	Oceanic phytoplankton >0.7 µm	-26.11 ± 0.36	2.88 ± 0.40	-26.11 ± 0.36	2.88 ± 0.40
20 – 63 µm POM	Blooming phytoplankton 20 – 63 µm	-19.36 ± 0.38	6.31 ± 0.15	No bloom	No bloom
	Non-blooming phytoplankton 20 – 63 µm	-23.9 ± 0.78	4.47 ± 0.96	-23.7 ± 1.26	5.85 ± 1.19
0.7 – 20 µm POM	Blooming phytoplankton 0.7 – 20 µm	-21.15 ± 0.31	6.71 ± 0.93	No bloom	No bloom
	Non-blooming phytoplankton 0.7 – 20 µm	-23.63 ± 0.28	6.37 ± 0.91	-24.42 ± 0.58	5.9 ± 1.06

Table B2. Summer and winter phytoplankton $\delta^{13}C$ and $\delta^{15}N$ values (‰) ± SD used in the MixSIR isotope mixing model program for each POM size class.

	Summer plankton group proportions											
Plankton size fraction (µm)	**Amphipod**	**Barnacle**	**Cadoceran**	**Copepod**	**Decapod**	**Egg sacs**	**Euphasid**	**Ostracod**	**Phytoplankton**	**Pteropod**	**Salp**	**Veliger**
63 – 125	0	0.006	0	0.07	0	0.009	0	0.05	0.85	0	0	0.013
125 – 250	0	0.07	0.07	0.39	0	0.05	0	0.02	0.37	0.005	0	0.019
250 – 500	0.02	0.03	0.35	0.45	0.003	0.07	0.008	0.01	0.05	0	0	0.004
500 – 2000	0.06	0.01	0.17	0.47	0.14	0.06	0.03	0	0.005	0	0.05	0.01

Table B3. Summer plankton group proportions for all size fractions. Groups that were collected with very low proportions for all size fractions are not included.

	Winter plankton group proportions							
Plankton size fraction (µm)	**Barnacle**	**Chaetognath**	**Copepod**	**Decapod**	**Fish larvae**	**Phytoplankton**	**Pteropod**	**Veliger**
63 – 125	0	0	0.01	0	0	0.82	0.02	0.15
125 – 250	0.004	0	0.37	0	0	0.54	0.006	0.07
250 – 500	0	0	0.94	0	0	0.05	0	0
500 – 2000	0	0.01	0.75	0.10	0.08	0.04	0	0

Table B4. Winter plankton group proportions for all size fractions. Groups that were collected with very low proportions for all size fractions are not included.

	Summer		Winter	
Size fraction (µm)	**Mean $\delta^{13}C$ (±) SD ‰**	**Mean $\delta^{15}N$ (±) SD ‰**	**Mean $\delta^{13}C$ (±) SD ‰**	**Mean $\delta^{15}N$ (±) SD ‰**
63 – 125	-18.16 ± 0.98	6.94 ± 0.93	-20.40 ± 2.32	8.56 ± 0.99
125 – 250	-19.14 ± 0.93	8.06 ± 1.05	-21.97 ± 1.17	10.67 ± 0.52
250 – 500 µm	-19.45 ± 0.98	8.81 ± 1.35	-22.13 ± 1.66	10.59 ± 0.60
500 – 2000 µm	-19.16 ± 1.15	8.39 ± 1.79	-21.49 ± 1.74	11.35 ± 1.24

Table B5. Summer and winter size-fractionated surface plankton mean $\delta^{13}C$ and $\delta^{15}N$ values (‰ ± SD).