The effects of shellfish aquaculture on chlorophyll-*a* in the North East Pacific Ocean

by

Helen Ford
B.Sc., University of Victoria, 2006

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Supervisory Committee

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Abstract

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Food production systems need to keep pace with the rising global population. Food from aquatic environments comes from both capture fisheries and aquaculture. Industrial fishing pressure has caused a global loss of more than 90% of large predatory fishes and 80% of the world's fish stocks are reported as fully exploited or overexploited. Global finfish, shellfish and aquatic plant aquaculture has been steadily increasing to meet the global demand for seafood. In British Columbia, aquaculture is primarily marine, with salmon and shellfish accounting for the majority of species cultured. Although shellfish aquaculture accounts for significantly less production and value compared to salmon aquaculture, the amount of foreshore dedicated to farming shellfish is nearly half (44%) the total area utilized by all aquaculture in the Province. Introduced Pacific oysters (*Crassostrea gigas*) (74%) dominate shellfish aquaculture in British Columbia. Pacific oysters are known to be very efficient generalist filter feeders that can grow faster and larger than native species. Extensive aquaculture is a form of aquaculture, where farmed animals feed exclusively on naturally occurring food in the surrounding water column. The goal of this research was to determine if there was a measureable depletion of phytoplankton around shellfish farms along the west coast of Canada and the United States. Chlorophyll-*a,* a pigment found within phytoplankton, was used as a proxy for phytoplankton abundance for this study. In field season one, two bays were studied, one exposed to shellfish culture (Westcott Bay) and one not exposed to shellfish culture (Fisherman Bay). The concentration of chlorophyll-*a* was measured in each bay at three locations at two depths (0.5 and 3 meters) and at two tidal heights (high and low). Chlorophyll-*a* concentration was found to be related to either depth or

tide, with location in a bay showing no difference in either of the bays studied. In addition to water column measurements, 100 Pacific oysters were placed at two locations within Westcott Bay Seafarm to test for local differences in oyster growth. The results from this experiment showed that Pacific oysters grown in the center of a shellfish farm were smaller than oyster grown at the farm's periphery. Field season two tested for spatial patterns between chlorophyll-*a* concentration and proximity to a shellfish farm in three different bays (Westcott Bay, Trevenon Bay and Gorge Harbour). A measureable depletion footprint of chlorophyll-*a* concentration was detected in the two sheltered shallow bays tested (Westcott Bay and Gorge Harbour), whereas no depletion footprint was detected in the exposed, deep bay (Trevenon Bay). Tide height played a significant role in predicating chlorophyll-*a* concentration in all three of the bays studied. These results suggested that some areas may be more suitable for shellfish culture than others. Taken together, this research demonstrated a measureable gradient of phytoplankton in sheltered shallow bays exposed to shellfish culture with depletion closest to the farm site, as well as greater oyster growth at the periphery of shellfish farms where phytoplankton would be predictably in greater abundance.

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Chapter 1

1.0 Introduction

1.1 Global Population and the Rise of Industrial Farming

The current global population is 6.9 billon people and is projected to increase to 9.1 billion people by 2050 (DESA 2009, Godfray et al. 2010). In order to maintain this exponential population growth, global food production systems need to keep pace with this rising global population. Food comes from both terrestrial (crop agriculture and livestock) and aquatic systems (fisheries and aquaculture). Approximately 95% of our food comes from industrial farming of terrestrial crops and livestock (agriculture). The Green Revolution started in the early 1960s when terrestrial farms introduced genetically engineered crop varieties, and increased the use of fertilizers, pesticides and mechanical irrigation to increase crop yields (Tilman et al. 2001, Tilman et al. 2002, Royal-Society 2009). In aquatic systems, unlike terrestrial systems, capture fisheries targeting wild food sources, are responsible for the majority of food production globally (Table 1)(FAO 2009). A new study suggests that mean trophic level catch statistics may not be representative of true mean trophic level biodiversity in the ocean, although fisheries catch statistics are currently the main system used for predicting trends in marine biodiversity (Branch et al. 2010). In 2001, Watson and Pauly suggested that declining global trends in wild fisheries were masked by China over reporting fishery catch statistics to the Food and Agriculture Organization (FAO) in the 1990s (Watson and Pauly 2001). Due to this concern, the FAO separated China's fisheries catch data from the rest of the world. Global catch fluctuates yearly, especially at the species level as some species are more affected by changing climate patterns. Given this yearly fluctuation, and when China was removed from the global statistics, there was a declining trend in global catch of 0.36 million tonnes/year between 1988 -2001, which stabilized over the past decade (Pauly et al. 2002, FAO 2009, 2010). On top of the decline of the global capture fisheries, the mean trophic level of fish species caught has been steadily

declining by 0.05-0.10 trophic levels per decade (Pauly et al. 2002). Industrial fishing pressure has caused a global loss of more then 90% of large predatory fishes (Myers and Worm 2003), and 80% of the worlds' fish stocks are reported as fully exploited or overexploited (FAO 2009). While capture fisheries has been declining, industrial finfish, shellfish, and aquatic plant aquaculture production has been steadily increasing worldwide (Table 1), to maintain the global demand for seafood (Naylor et al. 2000, FAO 2009, 2010). People have been sustainably farming aquatic environments for thousands of years. Historically, fish farming occurred primarily in Asia, where integrated aquaculture with livestock, crops, and fish was used to recycle nutrients. This type of fish farming utilized organic by-products to produce protein (e.g. carp and tilapia) while generating nutrients to feed the next crop generation (Shang and Costa-Pierce 1983). In North America, coastal First Nations communities maintained and harvested clam gardens, and evidence of these traditional clam gardens is still visible in the remnant shell middens located up and down the coast of British Columbia (Williams 2006).

The global aquaculture sector is dominated by Asian Pacific counties, specifically China, which accounts for 88.8% of global aquaculture production (FAO 2010). Since 1970, the aquaculture industry has maintained an average annual growth rate of 6.6% per year, although it is expected that the rate of increase in most regions will slow down over the next decade (FAO 2010). Aquaculture is set to overtake capture fisheries as a source of food fish (FAO 2009, 2010). In 2008, aquaculture accounted for 45.7% of the world's food fish supply (FAO 2010). Of that 59.9% was freshwater aquaculture, 32.3% was marine aquaculture, and 7.7% was brackish-water aquaculture (FAO 2010).

Excluding China, fisheries and aquaculture produce more than 75.5 million tonnes of human food per year, 78.6% of the global fish production (Table 1). The remaining 20.6 million tonnes is used for non-food products such as the production of fishmeal and fish oil used by the finfish aquaculture industry, ornamental purposes, bait, pharmaceutical uses, as well as direct feeding in aquaculture and livestock (FAO 2009, 2010).

Table 1: World fisheries and aquaculture production and utilization, excluding China (FAO 2009, 2010)

Production (million tonnes)	2002	2003	2004	2005	2006	2007	2008	2009
Inland Capture	6.5	6.5	6.5	7.2	7.5	7.7	8.0	7.9
Inland Aquaculture	7.1	7.8	8.9	9.5	10.2	11.0	12.2	12.9
Total Inland	**13.5**	**14.2**	**15.4**	**16.7**	**17.7**	**18.7**	**20.1**	**20.8**
Marine Capture	70.2	67.2	71.4	70.3	67.5	67.5	67.0	67.2
Marine Aquaculture	5.5	6.0	6.5	6.7	7.3	7.5	7.6	8.1
Total Marine	**75.8**	**73.3**	**77.9**	**77.0**	**74.8**	**75.0**	**74.6**	**75.3**
Total capture	76.7	73.7	77.9	77.5	75.1	75.2	74.9	75.1
Total aquaculture	12.6	13.8	15.3	16.2	17.5	18.5	19.8	21.0
Total Fisheries	**89.3**	**87.5**	**93.2**	**93.7**	**92.6**	**93.7**	**94.8**	**96.1**
Utilization (million tonnes)	2002	2003	2004	2005	2006	2007	2008	2009
Human consumption	66.2	68.1	68.8	70.4	72.4	73.5	74.3	75.5
Non-food (uses)	23.2	19.4	24.5	23.3	20.2	20.2	20.5	20.5
Total Utilization	**89.3**	**87.5**	**93.2**	**93.7**	**92.6**	**93.7**	**94.8**	**96.1**
Population (billions)	5.0	5.1	5.2	5.2	5.3	5.4	5.4	5.5
Per capita food fish supply (kg)	13.2	13.4	13.4	13.5	13.7	13.7	13.7	13.7

1.2 Marine Aquaculture

Marine aquaculture consists primarily of farming finfish, molluscs, crustaceans, and aquatic plants. Other animals are farmed at a much lower production volume (FAO 2010). Aquatic plants are not generally used directly as a source of food (except for edible seaweeds consumed primarily in Asia), but used more commonly for food additives, and extracts (Glicksman 1987). The most common species of marine finfish farmed are carnivorous Atlantic salmon *(Salmo salar)*, which are generally higher in value compared to other forms of aquaculture such as farmed aquatic plants and marine shellfish (mussels, oysters and clams) but much lower in quantity (FAO 2009, 2010).

1.3 Pelagic Ecosystems

Oceanographic environments are described by physical characteristics that differentiate vertical zones in the ocean. The ocean is divided into three main zones: pelagic, demersal and benthic. The pelagic zone characterizes the upper water column from the surface of the ocean to just above the seafloor. The demersal and benthic zones

of the ocean are described as the water column just above the seafloor and the seafloor. Within pelagic environments there are three main zones, the euphotic zone, the disphotic zone, and the aphotic zone (Letelier et al. 2004). The euphotic zone (or epipelagic zone) typically extends from the surface of the ocean to approximately 100 meters depth. The lower limit of this zone changes based on the maximum depth that can support photosynthesis (Letelier et al. 2004). Primary productivity is defined as the amount of carbon dioxide fixed by photosynthetic organisms within a given habitat. Different ecosystems have different environmental factors that govern its productivity, such as nutrient availability, available sunlight, upwelling, currents, and depth. Chlorophyll-*a* is the main pigment in phytoplankton that converts light energy into chemical energy during photosynthesis (Hoepffner and Sathyendranath 1991). In oceanography chlorophyll-*a* is commonly used as a proxy for quantifying the primary productivity or abundance of phytoplankton in a given area (UNESCO 1966, Hayward and Venrick 1982, Holm-Hansen et al. 2000). Chlorophyll-*a* is relatively easy to measure and has been linearly correlated with primary productivity and phytoplankton biomass (UNESCO 1966, Hayward and Venrick 1982, Holm-Hansen et al. 2000).

1.4 Human Introduction of Non-native Species

Anthropogenic introduction of marine non-native species is a growing concern (Wonham and Carlton 2005). Introduction can be both intentional (e.g. aquaculture) and non intentional (e.g. hull fouling, ballast water) (Carlton 1985, 1987, Naylor et al. 2001). An invasive or exotic species is any species that has become established outside the bounds of its native range (Molnar et al. 2008). An exotic species may disrupt ecological dynamics in a myriad of ways including the potential to outcompete native species for common food resources and habitat space (Ruesink et al. 2005). Successful invading species typically possess fast growth rates, high fecundity, and wide environmental tolerances (Ehrlich 1986, Ruesink et al. 2005). Non-native species are being introduced for the use of aquaculture worldwide (Naylor et al. 2001). These are an example of "controlled" introductions where exotic species are initially confined to a particular aquaculture site. Although these introductions are controlled in space (delineated by the farm boundaries) and time (when animals are introduced), they still have the potential to impact the allocation of resources available to native species in the surrounding

ecosystem (Ruesink et al. 2005). There are two broad categories of aquaculture: *intensive aquaculture* where farmed animals are solely dependent on imported nutrients such as commercial feeds, and *extensive aquaculture* where animals rely on food supplies from the natural environment (Little and Bunting 2005). Integrated aquaculture is an example where both *intensive* and *extensive* methods are employed in a single site. In systems of *extensive aquaculture* there is a net reduction of energy available in the system because the farmed animals sequester ambient food energy (Ruesink et al. 2005). Once the cultured animals reach market size they are harvested and the eco-energetic investment in the animal is lost to the system. Therefore, human introduction of *extensive aquaculture* could potentially impact the carrying capacity and energy flow within a given area (Jiang and Gibbs 2005, McKindsey et al. 2006).

1.5 Aquaculture in British Columbia

Aquaculture in British Columbia is divided into three main groups: (1) salmon which accounts for 89.8% production biomass and 94.2% value; (2) shellfish (oysters, clams, scallops and other) which accounts for 8.58% production biomass and 3.91% value; and (3) cultured other (including aquatic plants, plankton, freshwater trout, sablefish, sturgeon, and tilapia) which accounts for 1.40% production biomass and 1.90% value (MOE 2010). British Columbia is the fourth largest producer of cultured salmon in the world and currently cultures four species of salmon: non-native Atlantic salmon (95.2%) *(Salmo salar)*, and three species of native Pacific salmon (4.7%) (chinook, *Oncorhynchus tshawytscha,* coho, *Oncorhynchus kisutch,* and sockeye, *Oncorhynchus nerka)* (MOE 2010). All species of finfish, shellfish and aquatic plants that are currently cultured in the Province including species that are cultured in limited or experimental quantities are listed in (Table 2) (MAL 2010). Although aquaculture in British Columbia is dominated both in production and value by cultured salmon, shellfish aquaculture is characterized by significant year to year growth. In 1998, a Shellfish Development Initiative was released by the provincial government that aimed to double the Crown land available for shellfish aquaculture to 4230 hectares by 2008. Although that goal has not been met, the amount of land utilized by shellfish aquaculture and the number of sites compete with cultured salmon. The total area utilized by the aquaculture industry in British Columbia is 8110 hectares with salmon aquaculture utilizing 56.4% of that total

area, whereas the shellfish industry uses 43.6% of the total area, with dramatically lower economic yield per hectare. To further compound the comparison between the two industries, the shellfish industry currently occupies 508 sites, nearly four times the 131 sites dedicated to salmon aquaculture in the province. Not only is the shellfish industry in the Province utilizing a large percentage of marine foreshore for modest economic gain, it is dispersed over a greater area than other forms of aquaculture.

Table 2: Actively cultured species listing in British Columbia 2009 (*note: (*)varnish clams are not licensed for culture but licensed for harvest from aquaculture sites*) (adapted from (MOE 2010).

Finfish		**Shellfish**		**Marine Plants**	
Species	Scientific Name	Species	Scientific Name	Species	Scientific Name
Atlantic Salmon	*Salmo salar*	Abalone	*Haliotis kamtschatkana*	Kombu	*Laminaria saccharina*
Brook Trout	*Salvelinus fontinalis*	Nuttall's Cockle	*Clinocardium nuttallii*	Groenlandica	*Laminaria groenlandica*
Chinook Salmon	*Oncorhynchus tshawytscha*	Geoduck Clam	*Panope abrupta*	Giant Kelp	*Macrocystis integrifolia*
Coho Salmon	*Oncorhynchus kisutch*	Littleneck Clam	*Protothaca staminea*	Marine Micro-algae	*Gen spp*
Crayfish	*Pacifastacus leniusculus*	Manila Clam	*Tapes philippinarum*	Bull Kelp	*Nereocystis luetkeanna*
Kokanee	*Oncorhynchus nerka*	Varnish Clam*	*Nuttalia obscurata*		
Black Cod	*Anoplopoma fimbria*	Western Blue Mussel	*Mytilus trossulus*		
Sockeye Salmon	*Oncorhynchus nerka*	Eastern Blue Mussel	*Mytilus edulis*		
White Sturgeon	*Acipenser transmontanus*	Gallo Mussel	*Mytilus galloprovincialis*		
Tilapia	*Oreochromis niloticus*	Eastern Oyster	*Crassostrea virginica*		
Rainbow Trout	*Oncorhynchus mykiss*	Pacific Oyster	*Crassostrea gigas*		
		European Oyster	*Ostrea edulis*		
		Giant Rock Scallop	*Crassadoma gigantea*		
		Japanese Scallop	*Crassadoma gigantea*		

1.6 Shellfish Aquaculture

1.6.1 The British Columbia Situation

There are currently 508 licensed shellfish tenures (~3535 hectares) along British Columbia's marine foreshore (Figure 1). The two main species of shellfish cultured in British Columbia are Pacific oysters (*Crassostrea gigas*) (74%) and Manila clams (*Tapes philippinarum*) (16.4%). Goeduck clams (*Panope abrupta*), gallo mussels (*Mytilus galloprovincialis*) and scallops (*Crassadoma gigantean, Crassadoma gigantean*) are also cultured at a smaller scale and combined account for (9.4%) of the total shellfish produced in the province. Most shellfish aquaculture facilities in British Columbia have licences to culture multiple species of shellfish on the same farm. Since Pacific oysters make up the vast majority of cultured shellfish in British Columbia they are the main species of interest in this research although it is uncommon for aquaculture sites to farm only Pacific oysters. There are approximately 94 grams of oyster flesh per square meter of Pacific oyster farm (Banas et al. 2007), thus in 2009, there were approximately 24.6 x 10^5 kilograms of Pacific oysters being raised for human consumption in British Columbia's waters (MOE 2010).

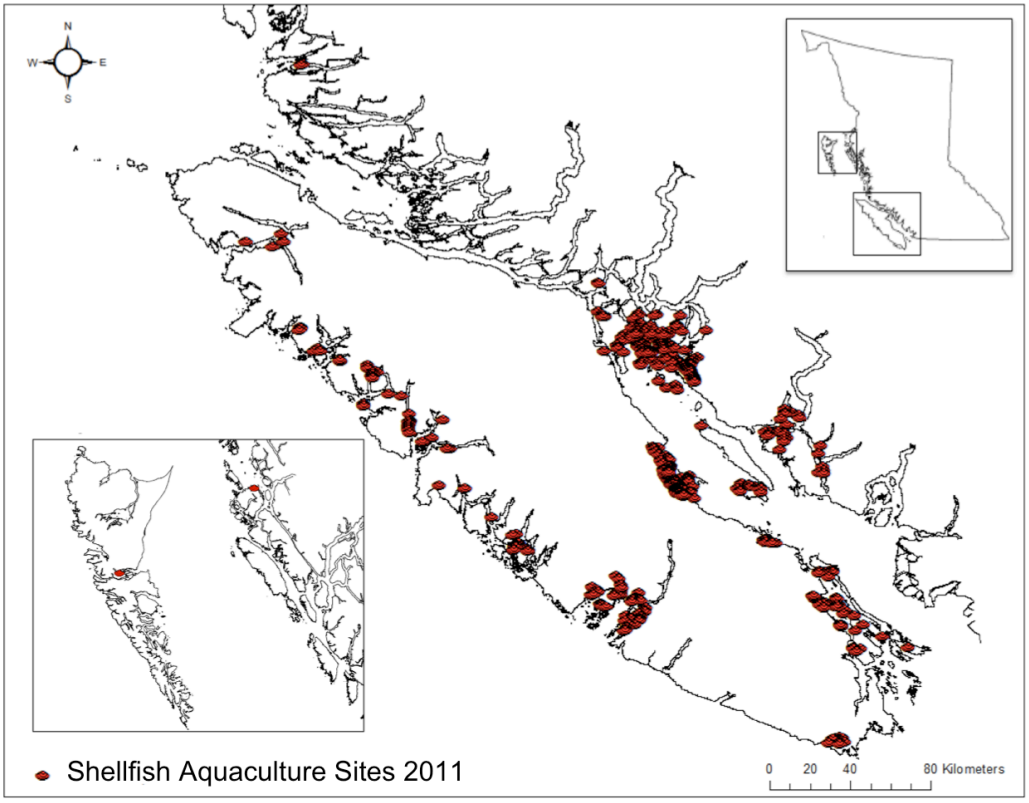

Figure 1: Shellfish Aquaculture sites in British Columbia (2011) (GEOBC 2010).

1.6.2 Pacific Oysters

The Pacific oyster (*Crassostrea gigas;* Family Ostreidae*)* is a marine bivalve that was introduced to the Pacific coast of North America (Pauley et al. 1988, Quayle 1988). The Pacific oyster is native to Japan and surrounding areas and was first introduced to Ladysmith Harbour and Fanny Bay, British Columbia (BC) for aquaculture in 1912 (Quayle 1988, Hamouda et al. 2004, BCSGA 2007). Since introduction to BC several areas have naturally established populations of Pacific oysters (BCSGA 2007). The Pacific oyster occupies the sub-tidal and the low to mid intertidal zone to a maximum depth of 3 meters, in temperate wave-protected areas (Pauley et al. 1988, Quayle 1988). Pacific oysters require water temperatures between 4-24 °C to survive and grow (Quayle 1988). Reproduction is via broadcast spawning where both eggs and sperm are produced and released into the water column and fertilization occurs externally (Quayle 1988). Spawning events are triggered by either a rise in sea-surface temperature, chemical cues or a combination of both (Quayle 1988). Mass spawning events enable synchronized

spawning en mass resulting in high concentrations of eggs and sperm in the water column at the same time to maximize successful fertilization (Quayle 1988). Successful spawning requires water temperatures to be between 20-23°C (Quayle 1988). Since it is rare for water temperatures to get this high along the coast of British Columbia, natural spawning events are rare (Quayle 1988). Fertilized eggs develop into larval veliger larvae (Quayle 1988). Once larvae reach a length of 0.30 mm and have spent three weeks as free swimming planktonic larvae, they settle and metamorphose from larvae to sedentary oysters (Quayle 1988). The presence of other oyster shells, and surfaces with irregularities stimulate oyster settlement (Pauley et al. 1988, Quayle 1988). Once an oyster larva has settled, it is referred to as "spat" (Pauley et al. 1988). Settlement usually occurs on a hard clean surface such as wood, reef, or bedrock (Quayle 1988).

Juvenile Pacific oysters are very susceptible to predation (Pauley et al. 1988). Dungeness crab (*Cancer magister)*, red rock crab (*Cancer productus)*, and graceful crab (*Cancer gracilis)* are known to chip and open juvenile oysters with their claws (Pauley et al. 1988). Sea-stars (sun star (*Pycnopodia helianthoides),* ochre star (*Pisaster ochraceus),* pink star (*Pisaster brevispinus)* and the molted star (*Evasterias troschelii)*) are the main predators of juvenile and adult Pacific oysters. Sea-stars attach to the oyster using their tube feet and can digest the whole organism using its eversible stomach (Pauley et al. 1988, Quayle 1988, BCSGA 2007). Oyster drills (Japanese drill (*Ceratostoma inornatum)* and eastern drill (*Urosalpinx cinerea)*) are introduced predators which prey on both juvenile and adult Pacific oysters. Drills are gastropods that have an extensible toothed rasping mouthpiece that can drill through the shells of oysters and other molluscs and feed directly on the flesh of the organism (Pauley et al. 1988, Quayle 1988, BCSGA 2007).

1.6.3 Farming Methods

There are two primary methods for farming Pacific oysters in British Columbia, beach culture and off-bottom culture (or a combination of both) (Quayle 1988, BCSGA 2007). Beach culture is a method where Pacific oysters are grown either directly on the sediment or in bags in the intertidal zone (Quayle 1988, BCSGA 2007). Beach cultured Pacific oysters are usually covered with anti-predator nets that exclude predators from large sections of the intertidal zone along the coast. Off-bottom culture is a method

where oysters are placed in large trays or nets or woven into lines which are suspended from rafts or long lines into deep water (Quayle 1988). Off-bottom methods increase the available habitat for Pacific oysters to grow in two primary ways: (1) moving from a 2-dimensional area (bottom culture) to a 3-dimensional volume (off-bottom culture) which increases the total number of animals per unit area (2) increasing the amount of time animals spend each day in the water column to 100% thus increasing the amount of time available to feed thereby shortening the total time needed for animals to reach market size. Sometimes a combination of both methods are used to get the benefits of both types of growing environments. Farmers want to maximize their profit so it makes sense to grow as many animals as possible in a short period of time – off-bottom culture maximizes space and growing time, but also has limitations. Animals that are grown entirely using the off-bottom methods have an increased mortality when they are harvested and taken out of the water for extended periods of time. This is because they have spent their entire life with their shell valves open in the water column feeding, whereas animals that are grown directly in the intertidal are subjected to natural tidal variation where they spend a portion of each tidal cycle exposed to the air. These animals have strong abductor muscles that lock the shell valves together with a small amount of seawater to allow animals to survive extended periods of the day exposed to air, terrestrial predators and variable temperatures. To maximize feeding time, and minimize harvest related mortalities, farmers often employ both methods of farming, first oysters are primarily raised in off-bottom culture to maximize space and growing time, once animals reach markets size they are then distributed in the intertidal, a process known to the industry as “hardening”.

1.6.4 Carrying Capacity

The introduction of large scale, extensive mono and multi-species aquaculture facilities to marine habitats changes the compliment of species in the near-shore marine ecosystem (Kelly et al. 2008) and potentially impacts biodiversity and energy flow (Ruesink et al. 2005). One method of quantifying biodiversity in a given area is to determine its species richness (Worm et al. 2006). Species richness refers to the number of species in a given area together with the relative abundance of each species (Cardinale et al. 2007). As the number of species and the number of individuals in each species

increases in a given area, the number of species interactions also increases (Ulanowicz 1997). Both strong and weak inter-specific and intra-specific interactions contribute to the stability and resilience of a system (Ulanowicz 1997). In general, systems that have more connections are more stable, and therefore more resilient to disturbance (Folke et al. 2004, Kinzig et al. 2006). Ecosystem services refer to the fundamental natural processes and natural resources that human civilizations depend on such as clean air and water (Folke et al. 2004). Ecosystems with high biodiversity are generally more stable, and offer more ecosystem services than systems with low biodiversity (Folke et al. 2004, Kinzig et al. 2006). Human activities such as the introduction of large and small scale aquaculture facilities to coastal environments is changing the compliment of species within local ecosystems. Introducing one or two numerically dominate species to an ecosystem changes the linkages within the ecological network eroding stability, resilience and ecosystem services (Folke et al. 2004).

Ecological carrying capacity in the current context is defined as the amount of aquaculture production that could be supported without significantly changing the major energy fluxes or structure of the food web in which the production system is embedded (Jiang and Gibbs 2005, McKindsey et al. 2006). *Ecological carrying capacity* is significantly different from *production carrying capacity*, which is the theoretical maximum aquaculture production that could be supported by the ecosystem (Jiang and Gibbs 2005, McKindsey et al. 2006). Aquaculture facilities are often unaware that they are reaching the *production carrying capacity* of an ecosystem until the high density farmed individuals experience reduced growth rates due to competition for food resources (Ruesink et al. 2005). Aquaculture sites that have reached, or are very close to the *production carrying capacity* could have severe ecological consequences, such as a reduction in suspended particulate food available to the surrounding near-shore marine ecosystem and spikes in benthic nutrient loads (Ruesink et al. 2005, Kelly and Volpe 2007).

1.6.5 Feeding Preference

Pacific oysters are very efficient conspicuous filter feeders (Dame and Prins 1998) and are much bigger and faster growing compared to British Columbia's native Olympia oyster (*Ostrea lurida*) (Quayle 1988, Ruesink et al. 2005, White et al. 2009). Its

faster growth rate and larger size is enabled by its higher filtering capacity and its ability to ingest a wider range of particle sizes (Quayle 1988). Oysters feed on planktonic organisms from the water column which are filtered by the gills and entrapped and bound in mucus (Cognie et al. 2001).

Oysters ingest bacteria, protozoa, phytoplankton, larval forms of other invertebrates and fish, and inanimate organic material (Pauley et al. 1988, Quayle 1988). Both phytoplankton and zooplankton are important food resources for Pacific oysters. The carrying capacity of a given ecosystem is dictated by its primary production. Variability in plankton biomass around shellfish farms could potentially impact energy at higher levels in the food chain.

Depletion of planktonic biomass is known to have large scale effects on ecosystem dynamics. For example in freshwater systems during the late 1980's and early 1990's there was a rapid explosion of zebra mussels (*Dreissena polymorpha)* in the Laurentian Great Lakes (Great Lakes) ecosystem. Zebra mussels were introduced via ballast water of ships and rapidly took over the benthic ecosystem (Bridgeman et al. 1995, MacIsaac et al. 1995). Soon after zebra mussels were established in the Great lakes, there were reductions in both the taxonomic composition of zooplankton and total planktonic biomass of the lake (Bridgeman et al. 1995, MacIsaac et al. 1995). The increased grazing pressure by zebra mussel caused the large decline in plankton biomass. This resulted in displacing native species and caused large scale ecosystem changes in the Great Lakes ecosystem (Bridgeman et al. 1995, MacIsaac et al. 1995).

Pacific oyster aquaculture potentially replicates this situation in the marine environment. It differs in that the number of farms and density of organisms is largely controlled by industry, but is similar in that high densities of introduced animals are feeding on ambient resources in the water column. A recent marine example looking at the ecosystem response from the removal of Pacific oyster rafts from a shallow tropical lagoon in Taiwan showed a significant positive change in phytoplankton biomass (g WW m^{-2}) 2.5 years before and 2.5 years after the rafts were removed (t-test= 3.04, $p<0.01$) (Lin et al. 2009). This increase in phytoplankton biomass may be attributed to a release from grazing pressure from cultured oysters (Lin et al. 2009).

In many ways shellfish aquaculture is a particularly attractive form of marine aquaculture compared to salmon aquaculture as it does not require exogenous inputs such as feed (BCSGA 2007). But, alternatively it has the potential to change the energy flow, and community composition of the near shore marine ecosystem. In summary, Pacific oyster aquaculture utilizes ambient resources in the water column (phytoplankton, zooplankton, and particulate organic matter) to produce high-density single and multi species cultures for human consumption. The patchy distribution and high intensity of Pacific oyster tenures may result in local depletion of plankton biomass. Both phytoplankton and zooplankton are directly and indirectly the primary forage of native and farmed marine shellfish and juvenile fish and are therefore of fundamental importance (Pauley et al. 1988, Quayle 1988, Landingham et al. 1998).

1.7 Thesis Objective

The goal of this research was to determine if there was a measurable depletion of phytoplankton around shellfish aquaculture sites along the west coast of Canada and the United States.

This research evolved over two field seasons. During the summer of 2008, one farm site was studied intensively as well as one corresponding reference site. This work is detailed in Chapter 2 which addresses to two specific questions:

(1) Does chlorophyll-a concentration change with increasing distance from a shellfish farm? Do chlorophyll-*a* gradients exist in the absence of farms?
(2) Does chlorophyll-*a* concentration change between high and low tide? Is the same pattern seen in sites with and without shellfish farms?
(3) Does position within a farm influence Pacific oyster growth?

The three main hypotheses that come out of these questions are: (1) chlorophyll-*a* abundance will be reduced in ecosystems exposed to oyster aquaculture and the amplitude of the reduction will be the highest closest to the farm. Shellfish contained within a farm do not move and therefore are only able to access water within the farm, therefore water within the tenure is more likely to be depleted of chlorophyll-*a* than water on the other side of the bay; (2) chlorophyll-*a* concentration will decrease with

decreasing tidal height. The longer the water residency time in the bay, the more time the shellfish have to deplete the abundance of phytoplankton in the bay; (3) oysters grown at the periphery of the farm will have larger growth rates then oysters grown in the center of the farm.

During the spring of 2009, the methodology was changed to answer a more general question about shellfish aquaculture. Due to the complex nature of marine ecosystems and increasing pressure by the provincial government to promote shellfish farming, Chapter 3 is dedicated to one basic question:

(1) Is it possible to measure chlorophyll-*a* depletion around shellfish aquaculture sites regardless of their size and oceanographic environment?

It was hypothesized that it would be possible to detect a measurable depletion footprint around shellfish farms and that the magnitude of depletion would be strongest around larger (sites that contain more shellfish) shellfish sites, as well as sites in less oceanographically active areas. Having a larger farm usually indicates having more animals in an ecological unit, the more animals feeding the larger the effect size. Areas that have more oceanographic activity such as currents, waves, and upwelling will have higher flushing rates, meaning the same parcel of water will spend less time in a given area. Water that spends less time in an area exposed to feeding shellfish will be less depleted than water that spends more time exposed to feeding shellfish.

Chapter 2

2.0 Vertical Point Sampling of Chlorophyll-*a* at a farm (Westcott bay) and a reference (Fisherman bay) site in the San Juan Islands, USA.

2.1 Introduction

The introduction of shellfish aquaculture facilities to naturally intact marine habitats changes the compliment of species in the near-shore marine ecosystem (Kelly et al. 2008). Farmed shellfish have the potential to alter the allocation of resources available to other species in the surrounding ecosystem (Ruesink et al. 2005). In this chapter, chlorophyll-*a* concentrations in two bays, one bay containing a shellfish farm (Westcott Bay) and one adjacent, geophysically similar reference bay (Fisherman Bay) that had no history of shellfish culture will be compared. Chlorophyll-*a* (main pigment found in phytoplankton) concentration was used as a proxy for phytoplankton abundance in each bay (UNESCO 1966, Hayward and Venrick 1982, Holm-Hansen et al. 2000). The growth of oysters was also compared at two locations in Westcott bay. These findings were compared to chlorophyll-*a* concentrations taken from the same two locations to determine if water column chlorophyll-*a* concentration predicts oyster size or if similar trends were observed in the water column and the growth of oysters.

2.2 Methods

2.2.1 Vertical Sampling

2.2.1.1 Site Selection

Two sites were selected, one farm site and one corresponding reference site in the San Juan Islands, WA, USA. Westcott Bay Seafarms located in Westcott Bay (N48°35’48.07”, W123°8’47.34”), was selected to be the farm site and Fisherman Bay (N48°30’50.62”, W122°55’4.03”) was selected to be the paired reference site (Figure 2).

Westcott Bay Seafarms has cultured Pacific oysters in Westcott Bay since 1980 (personal communication). Oysters are grown off-bottom in lantern nets suspended from buoys which are evenly dispersed over the farm's nine marine hectares. Each lantern net holds up to ~1500 adult oysters. The owners and managers have been very supportive of the research program and have provided information on production numbers and access to the site.

Fisherman Bay was chosen to be the only reference site to Westcott Bay as it shared the most similar hydrographic features to Westcott Bay and was the only bay within reasonable proximity to Friday Harbour Labs where the seawater samples were analyzed. Garrison Bay was considered as a possible second reference site to Westcott Bay but was discarded because of its small size and proximity and orientation to the farm site. Fisherman Bay is located on Lopez Island and is ~20 km from Westcott Bay. It is the most similar bay in the San Juan Islands in terms of size, depth, and exposure to Westcott Bay and does not have a history of shellfish aquaculture. Other hydrographic features such as the depth of the photic zone, the presence of stratified layers within the water column caused by differences in either temperature (thermocline) or salinity (halocline), current, wind, and the location of freshwater inputs to each bay, were not considered at either of the sampling sites. One potential source of error was the presence of a small marina in Fisherman bay, however Westcott Bay also experienced significant vessel traffic.

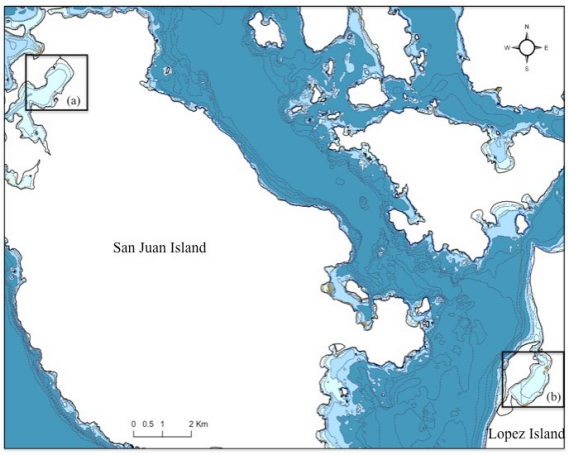

Figure 2: Map of study area showing the location of sampling sites used in 2008 (a) Westcott Bay (Farm site) and (b) Fisherman Bay (Reference site).

2.2.1.2 Chlorophyll a

Field Sampling

At each site (farm and reference) the concentration of chlorophyll-*a* was quantitatively determined at two tidal heights (high tide and low tide). Tides in North America are mixed semi-diurnal, therefore each tidal cycle was on average six hours apart (Banas et al 2007). Three discrete sample sites (A, B, and C) were used in each bay corresponding to the location of Westcott Bay Seafarms in Westcott Bay (Farm) and replicated in Fisherman Bay (Reference). The location of site A was near to the head of the bay, and site C was near the mouth of the bay, with site B occurring between site A and C (figure 3). At each of the three sample sites a 2 L messenger-activated horizontal water grab sampler was used to sample the water column at two discrete depths (0.5 m, 3.0 m). These depths were chosen to vertically sample the water column where farmed Pacific oysters were present. Two duplicate 250 mL sub-samples were taken from the 2 L sampler to determine the chlorophyll-*a* concentration at each depth in the water column. The chlorophyll-*a* samples were put on ice in a cooler and immediately covered with tinfoil to eliminate sunlight reaching them and stimulating further chlorophyll-*a* growth.

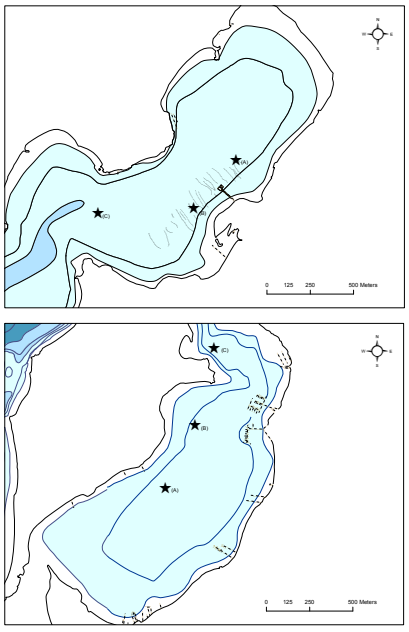

Figure 3: Map of study locations in 2008. Stars represent the location of sampling sites in both bays (A, B and C), (top) Westcott bay - black dashed lines indicate the location of Westcott bay Seafarms, (bottom) Fisherman bay.

Lab Analysis

All chlorophyll-*a* analyses followed extraction methods provided by The Institute of Ocean Sciences, Sidney, BC that were originally outlined in UNESCO 1966. The water samples were taken back to the lab and processed within one hour of collection in a dark room to reduce introducing error into the measurements. Once completed, the filter papers were then transferred to 20 mL scintillation vials ensuring the filter paper was green side up, and that the forceps never touched the sample. The scintillation vials were covered in tinfoil to protect the samples from light and then frozen at -80° C for no longer then 60 days to avoid sample degradation.

Chlorophyll-*a*, acetone preparation methods, chlorophyll-*a* extraction methods, and data processing methods were provided by Melanie Quenneville, phytoplankton technician, Institute of Ocean Sciences (IOS), Sidney, BC. Chlorophyll-*a* extraction was done at IOS by a certified analyst, Valerie Forsland.

Data Processing

Chlorophyll-*a* estimates were calculated following the procedure in JGOFS manual (1994). The basic equation used was as follows:

$$Chl(\mu g\ L^{-1}) = (F_m/F_m\text{-}1)\ x\ (F_0\text{-}F_a)x\ K_x\ x\ (Vol_{ex}\ /Vol_{filt})$$

F_m = acidification coefficient ($F_0/F_{a)}$ for pure chl (usually ~2)

F_0 = reading before acidification

F_a = reading after acidification

K_x = door factor from calibration calculations (use 1.0)

Vol_{ex} = extraction volume (usually 10 mL acetone)

Vol_{filt} = sample volume

2.2.1.3 Growth Experiment

A total of 1500 Pacific oysters that had just left the nursery were placed in three lantern nets (75 per tray times 10 trays per lantern times 2 lantern nets). The lantern nets were placed at two of the three sample sites (A, B) in Westcott Bay (Figure 3) and left to

grow for 1 year. One hundred oysters were randomly selected from the nursery racks and brought back to the lab. The oysters were gently cleaned using filtered seawater and a soft toothbrush. Once cleaned the oysters were placed in buckets of filtered seawater and left for 1 hour to clear their guts. The wet weight of each intact oyster (to 2 decimal places) was recorded as were shell morphometrics (maximum length, maximum width, and maximum depth) using digital callipers. Subsequently the flesh of each animal was separated from its shell and each were placed into different (one for the shell, one for the flesh) pre-weighted numbered weigh-boats. Both shell and flesh were then dried for 2 days at 60 °C and weighed (to 2 decimal places) to determine the oyster's dry weight.

One hundred oysters from each of the three lantern nets in Westcott Bay were re-sampled using the same protocol in early August 2009 (one year following their original placement). This was used to determine if there were any spatially explicit differences in Pacific oyster growth at the two sites and also to provide information about the rate of growth at the two locations (A and B).

2.2.1.4 Statistical Analysis

Chlorophyll a

ANOVA was used to test if the presence of a shellfish farm in a bay influenced chlorophyll-*a* concentration given three different parameters: (1) position in bay (A, B, and C), (2) depth (0.5, 3.0 meters), and (3) tide (high, low). The same parameters were also tested in a bay that did not contain a farm to see if any of the same patterns existed. The main parameter of interest in this study was position within the bay. It was predicted that the presence of a shellfish farm would influence the mean concentration of chlorophyll-*a* at the three different locations in the bay (A, B, and C) and that the concentration would be greatest at positions furthest away from the farm. Position A was closest to the head of the bay, position B was located in the middle of the bay (mid farm) and position C was located near the mouth of the bay. Duplicate water samples were collected at each of the three sample locations, at 0.5 and 3.0 meters, and at high and low tide in July, 2008. Taking duplicate water samples did not ensure precision in the sampling, and future sampling methods would employ taking triplicate samples for quality control of sample readings. If the absolute difference between duplicate samples was greater than two standard deviations from the mean difference between samples they

were removed (three samples were removed of 144, triplicate sampling would have eliminated this error in sampling). Sampling error or processing error were likely to have influenced samples that had large deviations between duplicate measurements. Once removed, the average of the two duplicate samples was used to compare mean differences in chlorophyll-*a* with the parameters of interest.

Growth experiment

The growth of Pacific oysters was measured at the two different sites (A and B) in Westcott Bay over a 12 month period. Oyster whole wet weight, dry tissue and shell weight were compared between the two sites using t-tests to investigate if there were any differences in oyster growth between the two sites. These results were then compared to the water column results of chlorophyll-*a* content at the same sites to investigate the link between available chlorophyll-*a* in the water column and oyster growth in Westcott bay.

2.3 Results

2.3.1 Vertical Sampling

2.3.1.1 Chlorophyll a

Westcott Bay (Farm site)

Duplicate chlorophyll-*a* samples were collected for six days in July, 2008 totalling 144 measurements. A cube root transformation was used to stabilize the variance and normalize the data. The maximal model (all parameters of interest and their interactions were included) was fit to the data using an analysis of variance test (aov) and model selection was employed to reduce the model to the best fit. Model selection was used to remove highest order non-significant terms from the model and AIC and likelihood ratio tests were used to test the new model fit. The best fit model, was the model that has the lowest significant AIC. The likelihood ratio test determined that there was no significant difference between the last two model iterations (SS=-0.28405, F=1.722, p=0.285) and therefore, the simplest model was selected as the best model. The results of the model selection show that *depth* was the only parameter that described differences in mean chlorophyll-*a* concentration in Westcott Bay (figure 4). The mean concentration of chlorophyll-*a* at the surface was 1.70 ug/L, and 2.16 ug/L at depth

(AIC=102.1531, F= 15.226, df=1, p<0.001) (Figure 4). Both *position in the bay* (A, B and C) (Figure 5) and *tide height* (high and low) (Figure 6) did not play a significant role in predicting chlorophyll-*a* concentration in Westcott Bay and were removed from the model.

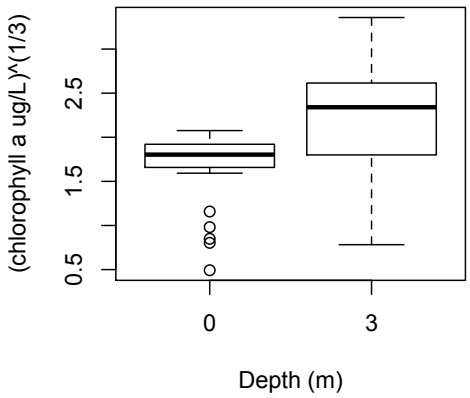

Figure 4: Boxplot comparing the transformed chlorophyll-*a* concentration at two different depths (0.5, 3.0 meters) in Westcott Bay. The heavy weighted horizontal line shows the median transformed chlorophyll-*a* concentration, the top and bottom of the box show the 25th and 75th percentiles, and the whiskers show 1.5 times the interquartile range of the data (approximately 2 standard deviations).

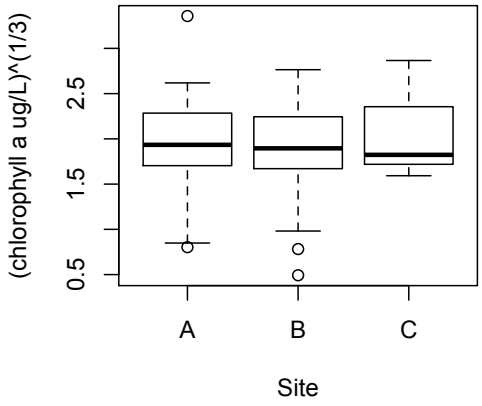

Figure 5: Boxplot of transformed chlorophyll-*a* concentration at the three sample sites (A, B and C) in Westcott Bay. The heavy weighted horizontal line shows the median transformed chlorophyll-*a* concentration, the top and bottom of the box show the 25th and 75th percentiles, and the whiskers show 1.5 times the interquartile range of the data (approximately 2 standard deviations).

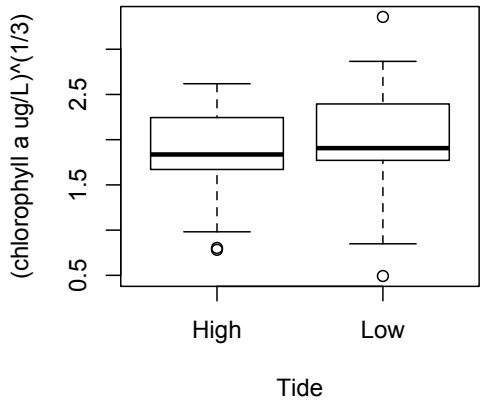

Figure 6: Boxplot of transformed chlorophyll-*a* concentration at high and low tide in Westcott Bay. The heavy weighted horizontal line shows the median transformed chlorophyll-*a* concentration, the top and bottom of the box show the 25th and 75th percentiles, and the whiskers show 1.5 times the interquartile range of the data (approximately 2 standard deviations).

Fisherman Bay (Reference site)

Fisherman Bay was selected as the reference site to Westcott Bay to compare the concentration of chlorophyll-*a* in a site that was not exposed to shellfish culture. Duplicate chlorophyll-*a* samples were collected over a period of six days in August 2008 totalling 144 measurements. A log transformation was used to stabilize the variance and normalize the data. The same statistical method used in Westcott Bay was used in Fisherman Bay. The maximal model (all parameters of interest and their interactions were included) was fit to the data using an analysis of variance test (aov) and model selection was employed to reduce the model to the best fit. Model selection was used to remove highest order non-significant terms from the model and AIC and likelihood ratio tests were used to test the new model fit. The best fit model, was the model that had the lowest significant AIC. The results of the model selection indicated that the model that included both *tide* and *depth* was the best fit (lowest AIC), however, when compared using a likelihood ratio test to the further reduced model, the test showed no significant difference (SS=-0.90426, F=3.1818, p=0.079) between the lowest AIC model (*tide* and *depth)* (AIC= 118.6804) and the further reduced model (*tide* only*)* (AIC= 119.9263) indicating that the simpler, further reduced model was not significantly worse and

therefore was selected as the best model (F= 8.0544, df=1, p=0.0059) (Figure 7). Both parameters *position in the bay* (A, B and C) (Figure 8) and *depth* (0.5, 3.0 m) (Figure 9) did not play a significant role in predicting chlorophyll-*a* concentration in Fisherman Bay and were not included in the best fit model.

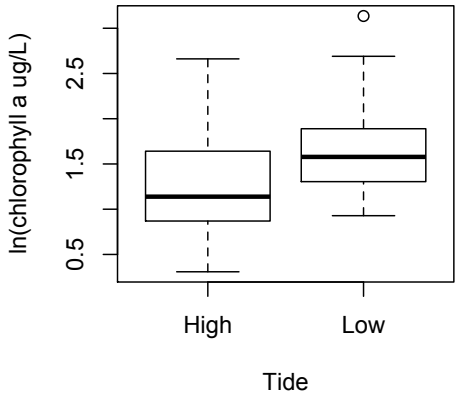

Figure 7: Boxplot comparing the transformed chlorophyll-*a* concentration at two tide heights (high, low) in Fisherman Bay. The heavy weighted horizontal line shows the median transformed chlorophyll-*a* concentration, the top and bottom of the box show the 25th and 75th percentiles, and the whiskers show 1.5 times the interquartile range of the data (approximately 2 standard deviations).

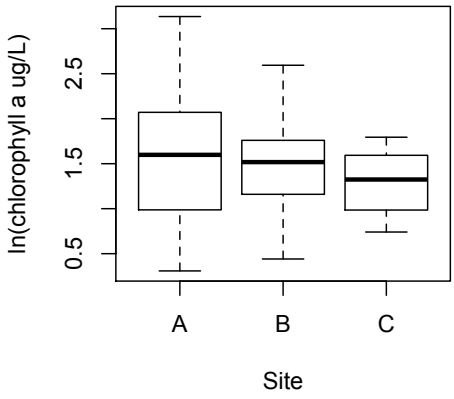

Figure 8: Boxplot comparing the transformed chlorophyll-*a* concentration at three sample locations (A, B and C) in Fisherman Bay. The heavy weighted horizontal line shows the median transformed chlorophyll-*a* concentration, the top and bottom of the box show the 25th and 75th percentiles, and the whiskers show 1.5 times the interquartile range of the data (approximately 2 standard deviations).

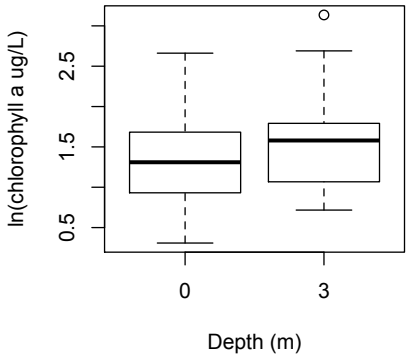

Figure 9: Boxplot comparing the transformed chlorophyll-*a* concentration at two different depths (0.5 and 3.0 meters) in Fisherman Bay. The heavy weighted horizontal line shows the median transformed chlorophyll-*a* concentration, the top and bottom of the box show the 25th and 75th percentiles, and the whiskers show 1.5 times the interquartile range of the data (approximately 2 standard deviations).

2.3.1.2 Growth Experiment

Pacific oyster growth was compared between two sites in Westcott bay over a period of 12 months. The difference in growth of 100 Pacific oysters placed at two sites (A and B) in Westcott bay were compared. Growth was measured in three ways: *whole wet weight (g)*, *dry flesh weight (g)*, and *dry shell weight (g)*. The distribution in size of Pacific oysters followed a normal distribution in each of the three measures of growth. A parametric t-test was used to test for differences in mean weight between the two sites. The variance between the two sites (A and B) was calculated for each of the three measures of growth, there was no significant difference in variance for oyster *whole wet weight* (F=1.4057, df=99, p-value=0.092), but *dry weight* and *shell weight* were significantly different (F=1.5094, df=99, p-value=0.042; F=1.5897, df=99, p-value=0.022). Therefore a t-test assuming equal variance was used for *whole wet weight* and t-tests assuming unequal variance were used for *dry weight and shell weight*. The results of the t-tests showed the same trend across all three measures of growth (Table 3). Oysters grown at site A were significantly heavier than oysters grown at site B. The mean whole wet weight of oysters at site A was 90.37 +/- 37.83 g compared to 75.16 +/- 31.91g at site B (t=3.0735, df=192.5, p-value=0.0024). The mean dry weight of oysters as site A was 3.21 +/- 1.36 g compared to 2.29 +/- 1.11 g at site B (t=5.2589, df=190.163, p-value<0.001). The mean shell weight of oysters at site A was 50.66 +/- 22.20 g compared to 39.96 +/- 17.61g at site B (t=3.7779, df=188.241, p<0.001) (Figure 10).

Table 3: Summary table showing the results from three t-tests comparing differences in three measures of growth (g) (whole wet, dry flesh, and dry shell) between two sites (A and B) in Westcott Bay.

Measure of Weight (g)	***n***	**t Statistic**	***df***	**p-value**
Whole wet	100	3.0735	192.5	0.0024
Dry flesh	100	5.2589	190.163	<0.001
Dry shell	100	3.7779	188.241	<0.001

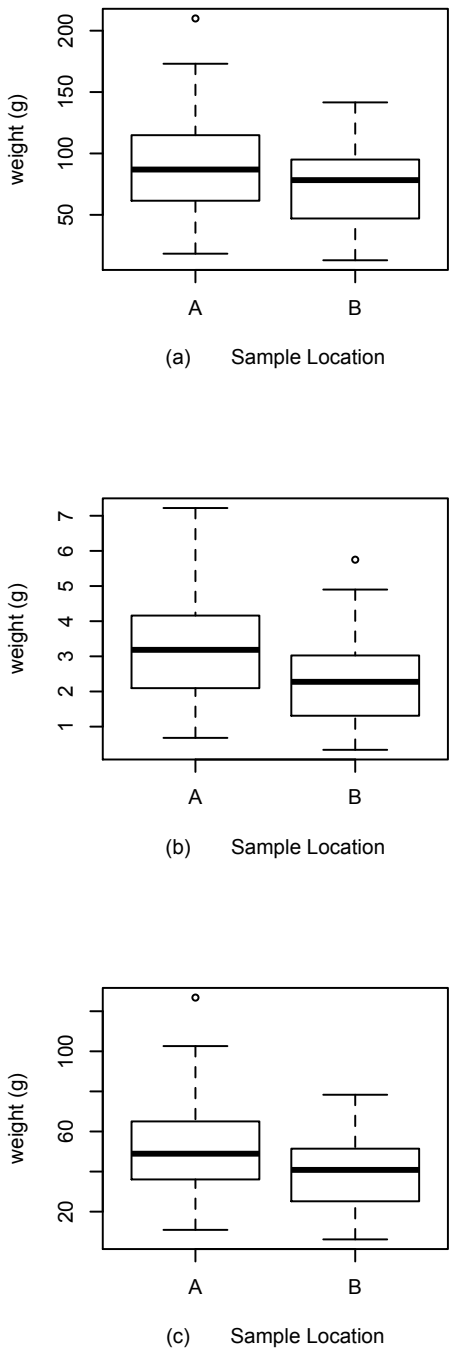

Figure 10: Boxplot comparing oyster growth at two sites in Westcott Bay (A and B). (a) mean difference in whole wet weight (g) between site A and B, (b) mean difference in dry flesh weight (g) between A and B, (c) mean difference in shell weight (g) between A and B. The heavy weighted horizontal line shows the median oyster weight (g), the top and bottom of the box show the 25th and 75th percentiles, and the whiskers show 1.5 times the interquartile range of the data (approximately 2 standard deviations).

2.4 Discussion

The results of the vertical point sampling study comparing the mean chlorophyll-*a* concentration at different locations in a bay, at two depths (0.5, 3.0 m), over two tidal heights (high, low) indicated that position does not predict the chlorophyll-*a* concentration in either Westcott Bay (Farm) or Fisherman Bay (reference). This result goes against the hypothesis and indicates the presence of the shellfish farm in Westcott Bay does not reduce phytoplankton abundance in a way that is measurable using vertical point sampling. It is also possible that phytoplankton maybe not be a limiting resource in the middle of summer and the effect from feeding oysters is not possible to detect. It is known that phytoplankton distribution is patchy and tends to aggregate together to form layers in the water column (Kiorboe and Hansen 1993). Therefore the use of vertical point sampling may not be the best tool to detect differences in the concentration of chlorophyll-*a*.

This method did detect significant differences in the mean chlorophyll-*a* concentration between Westcott Bay and Fisherman Bay. The chlorophyll-*a* concentration in Westcott Bay was found to be significantly higher at depth compared to the surface, whereas in Fisherman Bay the concentration was found to be significantly higher at low tide. It is impossible to say that the factors that lead Westcott Bay to have significantly higher chlorophyll-*a* concentration at depth are similar to the factors that lead Fisherman Bay to have significantly higher chlorophyll-*a* concentration at low tide since the hydrological similarities and differences between the two bays is not known. The purpose of this study was to determine if proximity to a shellfish farm decreased chlorophyll-*a* concentration in near-shore marine ecosystems. No horizontal spatial trends were detected from water column samples, however the results from the growth experiment indicated that oysters grown at site B were significantly smaller than oysters grown at site A. Site B is located in the middle of the shellfish farm, whereas site A is located on edge of the shellfish farm (nearest the head of the bay). Since the oysters were not sub-sampled over the course of the 12 month growing period, it is impossible to determine when this difference in size occurred. These results suggest that competition for food resources by farmed Pacific oysters may be present in Westcott Bay, and that food resources in the center of the farm may be in higher demand then elsewhere in the

bay. Future work should focus on mapping chlorophyll-*a* concentration in the bay to determine if there is a spatial pattern between chlorophyll-*a* and Westcott Bay Seafarms.

Chapter 3

3.0 Spatial patterns between chlorophyll-*a* and shellfish farms along the west coast of Canada and the United States.

3.1 Introduction

Farmed shellfish feed on ambient resources in the water column. Therefore, they have the potential to impact the allocation of resources available to other species in the surrounding ecosystem (Ruesink et al. 2005). Once the farmed shellfish reach market size they are harvested and the eco-energetic investment in the individual is completely lost to the system. In this chapter three bays that are fundamentally different will be compared to determine if it is possible to measure a footprint of chlorophyll-*a* depletion around a shellfish farm regardless of the number of farms in the bay, the farm size, or the bays oceanographic environment. Three spatial models that best parameterize the factors contributing to the concentration of chlorophyll-*a* in each bay will be developed. The models will then be used to predict chlorophyll-*a* concentration in each bay under similar circumstances. The results of these models will then be displayed on a map of each bay to visually represent the spatial patterns observed.

3.2 Methods

3.2.1 Horizontal Sampling

3.2.1.1 Site Selection

Three farm sites were selected for horizontal sampling; Westcott Bay, San Juan Islands, WA, USA, Trevenon Bay and Gorge Harbour, British Columbia, Canada (Figure 11). Westcott Bay (N48°35’48.07”, W123°8’47.34”), was chosen as a site because it

was used as the farm site during field season one (pilot study), and because of its proximity to the University of Victoria (UVic). The owners and managers of Westcott Bay were also very supportive of the research program by providing access to the site and in-kind support when needed, such as access to the dock, and use of the harvesting raft. Trevenon Bay (N50°1”25.57’, W124° 44’32.77”), located within Okeover Inlet was chosen as a site because of its high density of shellfish rafts. Seven off-bottom shellfish farms line both sides of the bay leaving only an opening down the center of the bay free from floats and shellfish rafts. Although Trevenon Bay was very different from both Westcott Bay and Gorge Harbour in terms of depth, size of bay and amount of shellfish culture, it was chosen because the bay has one of the highest densities of shellfish farms in British Columbia and created a novel opportunity to compare not only different oceanographic areas, but also areas with differing intensities of culture. Gorge Harbour (N50° 5’ 51.94”, W124°59’8.31”) located on Cortes Island was chosen as the third sampling site. The NE corner of Gorge Harbour was also larger in size than Westcott Bay which made it impossible to sample the entire harbour. Gorge Harbour was chosen as a sampling site because it supported shellfish culture sites and the mouth of the harbour was very narrow. Gorge harbour was surrounded by homes, a marina and was one of the only locations in the area with safe mooring available. The NE corner of the harbour was selected because it was out of the way of boat traffic, it was possible to navigate around the floats, and it was one of the larger farms within the bay. All three of the sampling locations had both bottom and off-bottom culture and cultured more than one species of shellfish – common to all sites is Pacific oyster culture, although the proportion dedicated to Pacific oyster culture at each location is unknown.

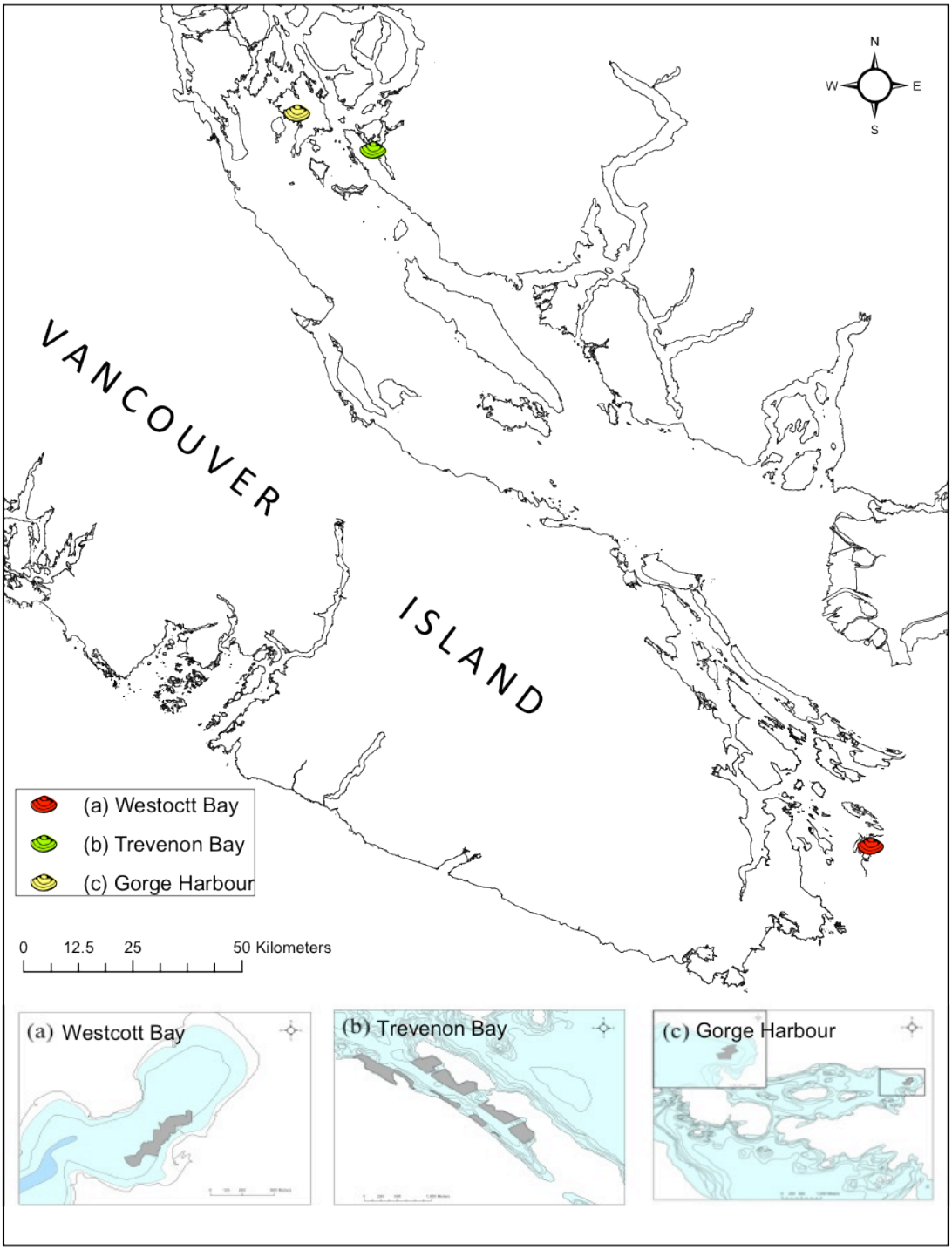

Figure 11: Map of study locations in 2009: (a) Westcott Bay, (b) Trevenon Bay, (c) Gorge Harbour. Grey polygons represent the size and location of shellfish culture at each location.

3.2.1.2 Water column sampling

At each of the three sampling sites (Westcott Bay, Trevenon Bay, and Gorge Harbour) the water column was sampled horizontally using a YSI 6600 data sonde attached to a Lowrance GPS (Table 4). The YSI 6600 data sonde is a submersible device that instantaneously measures characteristics of the water column and digitally records them at a given interval. The parameters measured for this research were: chlorophyll-*a*, turbidity, dissolved oxygen, conductivity (salinity), temperature, and depth. The main parameter of interest used in this research was the *in-situ* chlorophyll-*a* concentration. The sensor used on the sonde was the YSI 6025 wiped chlorophyll-*a* sensor. This sensor was designed to estimate chlorophyll-*a* concentration by instantaneously detecting algal fluorescence. The sensor emits a blue light (centered around 470 nm) and measures the fluorescence of all species in the water column. Fluorescence from chlorophyll-*a* in phytoplankton is reflected back at the chlorophyll-*a* sensor and is recorded. The detection range of the sensor is 0 - 400 ug/L with a detection limit of 0.1 ug/L and a resolution of 0.1 ug/L. A Lowrance Global Positioning System (GPS) equipped with both GPS and Wide Area Augmentation System (WAAS)) was attached to the sonde so that every measurement made in the water column was geo-referenced with a corresponding latitude and longitude as well as the time each measurement was recorded. WAAS increases GPS accuracy to a range within 7.6 meters vertically and horizontally and according to the Federal Aviation Administration typically comes within 1-2 meters horizontally and 2-3 meters vertically. This enabled water column measurements from each transect to be superimposed on a map of the bay so it would be possible to calculate the distance between each data point and a shellfish farm. Tidal height (meters) and tidal direction (ebb(+), flood(-)) were manually added to each of the water column measurements. The tidal heights were provided from the Pacific Region of the Canadian Hydrographic Service in one minute intervals specific to each of the sampling sites.

Table 4: Summary table of 2009 sampling statistics. *(note: (*)* Trevenon Bay statistics include transect 20 which was shortened due to bad weather).

	Total No. of Transects	Total No. of samples	Total Distance (m)	Avg time/ transect (min)	Avg distance/ transect (m)	Avg speed/transect (km/hour)
Westcott Bay	23	69386	124758.8	100.02 +/- 12.98	5424.3 +/- 670.8	3.27 +/- 0.24
Trevenon Bay *	22	62201	110176.1	95.44 +/- 20.79	5008.0 +/- 1015.69	3.17 +/- 0.16
Gorge Harbour	35	57500	105513.4	54.94 +/- 9.38	3014.7 +/- 537.1	3.29 +/- 0.13

The data sonde and GPS were then deployed using a floating raft (90 cm x 150 cm x 10 cm) allowing the sonde to sit 0.5 meters perpendicular to the surface of the water column. The raft, GPS and sonde were then towed 5 meters behind a 19 foot aluminum boat at slow speed. The speed of the boat was maintained at approximately 3 km/hour throughout each transect. The sonde was programmed to take a water column sample every two seconds. Similar methodologies using autonomous underwater vehicle's, or undulating mobile devices that measure *in-situ* chlorophyll-*a* concentration have been used in other studies (Barth and Bogucki 2000, Blackwell et al. 2008, Grant et al. 2008).

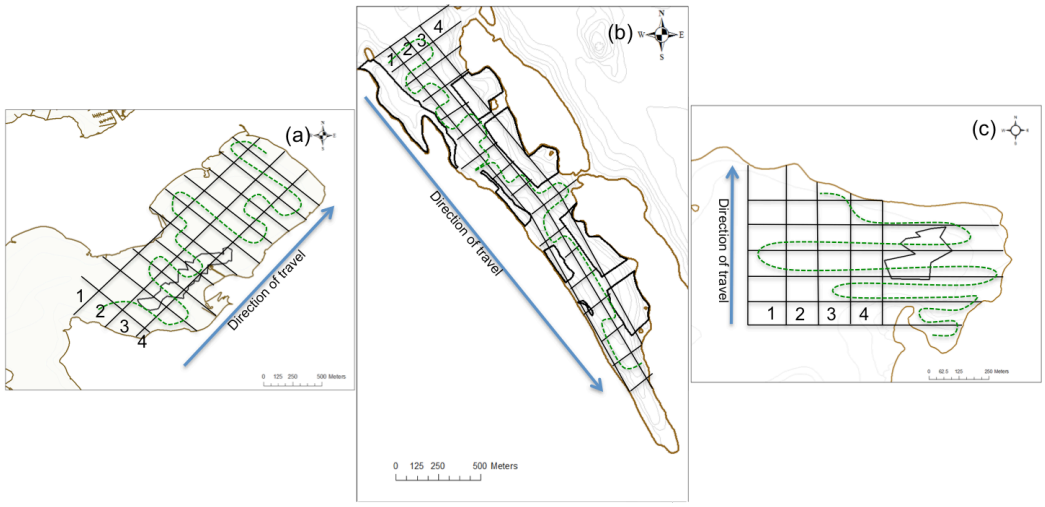

Figure 12: Schematic diagram of sampling methodology used in (a)Westcott Bay (b)Trevenon Bay (c) Gorge Harbour. The arrow marks the direction of sampling from mouth to head of the bay, dashed line represents an hypothetical transect of the bay following the correlated random walk study design using the grid pattern superimposed on the bay.

Westcott Bay was systematically divided into a (4 x 16) grid pattern. Columns (parallel lines running from mouth to head of the bay), and rows (perpendicular to the columns running from mouth to head of the bay) of the gird were assigned a number (Figure 12). A random number generator was used to generate a number between (1 - 4) to determine the path of each transect, a correlated random walk sample design, from the mouth of the bay to the head of the bay. The path of the transect was correlated because the route always started at the mouth of the bay and moved in the direction of the head of the bay (Figure 12). In Trevenon Bay it was not possible to drive the boat between rafts. This changed the sampling methods for this site as only the narrow section running up the middle of the bay could be sampled (Figure 12). Apart from not being able to access the water column within the farms, the methods used in Westcott Bay and Trevenon Bay were the same. In Gorge Harbour only the NE corner of the harbour was sampled (Figure 12). Since it was not possible to sample all of Gorge Harbour, the sampling methodology was modified. Instead of making a grid of the entire harbour, a grid that extended west towards the middle of the harbour (Figure 12). The shellfish farm within this corner of the harbour used rafts instead of floats making it harder to navigate around that farm, therefore the farm was not included in the grid and instead the same transect path within the farm was used for each transect. The random number generator between (1-4) was used to determine how far into the harbour to sample. Each sample transect in Gorge Harbour was correlated south to north instead of mouth of the bay to head of the bay. The correlated random walk sampling design was employed to reduce user bias on the path of each transect and to maximize the sampling area of each bay.

At each of the sites, the data sonde and GPS were connected to a data display and logging system (YSI 650 MDS) using a 6-series field cable. This allowed measurements to be observed in real time and minimized the amount of time the sonde was fouled with debris during field collection, reducing sampling error. The sonde recorded chlorophyll-*a* concentration every two seconds. The start and end time of each transect was recorded along with any times when the transect had to be stopped and started (i.e.: when one of the sensors was fouled, or if the GPS ran out of power). At the end of each sampling day, the results were uploaded for future analysis using EcoWatch software. At the beginning of each sampling day, the 6025 wiped chlorophyll-*a* sensor was calibrated in

de-ionized water until the reading stabilized at 0 ug/L. This ensured all chlorophyll-*a* data were calibrated to the same zero and thus directly comparable. The boundaries of each of the shellfish farms within the sampling locations was recorded with the GPS and saved as a separate file.

3.2.2 Statistical Methodology

Data from each of the three bays was compiled into three separate databases. Each measurement was assigned a unique identification number and coded to the specific date and transect where each measurement was taken. Both the water column measurement and GPS location were required for the data to be usable. Since tide height and tide direction were not measured by the data sonde, both these measurements were input into the databases manually. Tide height was reported every minute whereas water column parameters were recorded every 2 seconds by the data sonde. Therefore, for every 30 water column measurements there was only one corresponding tide height recorded. Tide direction was reported as either "ebb" (falling) or "flood" (rising). The databases were then saved as .DBF files and imported into ArcGIS software along with the location of each of the shellfish farms (Environmental Systems Resources Institute 2008). Using the ArcGIS software, polygons delineating the total area of each shellfish farm were created for each of the three sample bays (Westcott Bay, Trevenon Bay, and Gorge Harbour). Before calculating the distance between each water column measurement and the shellfish farm the projection of the data points was changed to enable measurement to be made in meters. The projection was changed from the geographic projection of NAD1984 to a UTM projection UTM10N. The NEAR function within the Arc software was then used to calculate the nearest distance between each of the water column measurements and the perimeter of the farm (or closest farm) in each of the three bays sampled. If the sample point was located within a farm polygon, it was assigned a negative value. In Trevenon Bay there were seven shellfish farms around the perimeter of the bay which may have caused the farm signal to be lost or dulled due to the inability to sample within the farm boundaries. Therefore, in Trevenon Bay the distance between each of the sample points and the mouth of the bay was also calculated

to test if there was a serial dilution effect occurring from mouth to head of the bay. Once all distance data was calculated, the data was exported back out of the ArcGIS software.

Completed data files for each of the bays were then imported into the statistical software R (R Development Core Team 2011). A linear mixed effects model fit by maximum likelihood was used to test if there was relationship between chlorophyll-*a* concentration and distance to a shellfish farm. The mixed effects model accounted for the hierarchical structure within the data. Mixed effects models have both fixed and random effects. The fixed effects parameters: *distance to shellfish farm (m), tide height (m)*, and *tide direction (ebb, flood),* were the parameters of interest in the study. Due to the nature of the study design, the models also included a random effect to account for the variance-covariance structure in the data. Since the same bay was repeatedly sampled over a period of six days, each data point was nested within transect, and each transect was nested within sample day. How chlorophyll-*a* concentrations changed transect to transect, or day to day, was not of interest in this study. A random effect term that incorporated for this nested structure was used to account for differences arising from samples collected on the same day or from the same transect. Within mixed effects models, it was also possible to account for temporal auto-correlation between data points. Since water column measurements were collected every two seconds it can be assumed that there was an element of temporal auto-correlation in the data, meaning data points collected near to each other in time were correlated. The sonde was towed at approximately the same speed in each transect, thus the data points were equidistant. To account for temporal auto-correlation in the data, an auto-regressive level 1 (AR(1)) correlation structure was applied to the mixed model.

Two mixed effects models were run for each bay. The first model included all the fixed effects parameters of interest: *distance to shellfish farm (m)*, *tide height (m)*, and *tide direction (ebb vs. flood)*, as well as the random effect parameter that accounted for repeated measurements taken in the same bay: 1/date/transect (the one indicates the random effect was on the intercept), as well as the auto-regressive level 1 correlation structure (AR(1)). The second model eliminated the fixed effect parameter *distance to shellfish farm (m)* and kept everything else the same. Model selection using AIC and a likelihood ratio test determined the best model for each bay. The best fit model, was the

model that had the lowest significant AIC. The likelihood ratio test compared the fit of the two models by calculating a ratio that describes how many times more likely the data fit one model over the other. This type of model selection specifically addressed if the parameter, *distance from shellfish farm,* significantly increased the model fit. Once the best model for each of the bays was determined, the PREDICT function, in the statistical software *R,* was used to predict the chlorophyll-*a* concentration at different distances from the shellfish farm(s). This enabled other parameters in the model to be held constant while only changing the parameter of interest (in this case – *distance from a shellfish farm*). The resulting maps are a visual representation of a multidimensional model that best fits the data for each of the bays sampled.

3.2.3 Mapping Methodology

To create a visual representation of the data, chlorophyll-*a* gradient maps of predicted values from the best fit mixed effects models were created for each of the three sample bays (Westcott Bay, Trevenon Bay and Gorge Harbour). Within each of the three bays, a layer of empty geo-referenced points at different distances from the shellfish farm(s) was created in ArcGIS. The NEAR function within the ArcGIS software was used to calculate the distance each water column measurement was from the shellfish farm. This created a data sheet that had distance measurements with corresponding latitude and longitude. Using the best fit model for each of the three bays, the PREDICT function in the statistical software *R* was used to calculate values of chlorophyll-*a* using the *distance from shellfish farm* measurements previously calculated from the empty geo-referenced data points layer in ArcGIS. Other parameters in the model such as *tide height* and *tide direction* were held constant and the new data file of predicted values was then saved as a .DBF file and imported it back into ArcGIS. Within the ArcGIS software the Inverse Distance Weighted (IDW) function was used to interpolate values between sampling points to get a smooth visual representation of chlorophyll-*a* concentration at different distances from a shellfish farm at three different tidal heights for both ebb and flood tide. IDW interpolated missing chlorophyll-*a* concentrations using surrounding known concentrations within a defined area. Known chlorophyll-*a* concentrations located nearest the missing concentrations were weighted more heavily then

concentrations further away. This method assumes that chlorophyll-*a* concentration within a given area is similar, which was supported by the high correlation coefficient (phi) provided by the mixed model.

2.3 Results

2.3.1 Horizontal Sampling

2.3.1.1 Westcott Bay

The best fit linear mixed effects model to describe chlorophyll-*a* concentration in Westcott Bay included all the fixed effects parameters of interest: *distance from farm*, *tide height*, and *tide direction* (Table 5). The likelihood ratio test to compare the two models showed that the best fit model was significantly better fit than the model that did not include the parameter *distance from farm* (L.Ratio=57.91, p-value <0.001, Table 5). The correlation coefficient (phi) between successive measurements showed measurements were highly correlated phi=0.973, and the auto-regressive level 1 correlation structure in the model was needed. The parameter estimates of the fixed effects for the best fit model are shown in Table 6 (estimates are for the (ln+1) transformed chlorophyll-*a* concentration). The relationship between distance from farm and chlorophyll-*a* concentration is shown in (Figure 13), each panel indicates a specific sampling day, each regression line within a panel indicates one transect (1-4), transect number represents successive transects completed on the same day. The figure shows that most transects have a slight positive slope with increasing distance from a shellfish farm. Figure 14 pools all the data from each transect together and looks at the overall trend in the data with respect to distance from farm separated by tide direction (flood, ebb). The data are variable, but the same daily trend shown in Figure 13 was observed for both flood and ebb tide. A negative relationship was observed between chlorophyll-*a* concentration and tide height that was more obvious during ebb tide (Figure 15).

It was hard to pull trends out of the raw data since all the parameters of interest were being observed under different conditions, for example, transect two on day one the tide may have been flooding with a tide height changing from 3.0 to 3.5 meters where as transect two on day six may have occurred on an ebb tide with a tide height changing from 0.0 to -0.5 meters. Therefore the maps provide a very useful tool to extract

biological significance from the model results. The maps of predicted chlorophyll-*a* concentration in Westcott Bay show the concentration of chlorophyll being the highest at low tide with a very small difference between flood and ebb tide. The concentration of chlorophyll-*a* was slightly less on a flood tide than an ebb tide. It was also obvious from the maps that *tide height* controls the concentration of chlorophyll-*a* more than the effect seen from the presence of a shellfish farm. On a flood tide, the concentration of chlorophyll-*a* changed from 1.34 ug/L at high tide to 3.33 ug/L at low tide, whereas the maximum difference in chlorophyll-*a* observed across the bay on a flood tide was 0.67 ug/L (Figure 16).

Table 5: Fixed effects of each model tested in each sample bay (Westcott Bay, Trevenon Bay and Gorge Harbour). Column *n* is the total number of water column measurements in each model, Phi is the correlation coefficient, *df* represents the degrees of freedom showing the number of parameters tested in each model, AIC is the Akaike's Information Criterion, L.Ratio is the corresponding likelihood ratio test comparing successive models.

Fixed Effects	*n*	Phi	*df*	AIC	L. Ratio	p-value
Westcott Bay						
Distance From Farm + Tide Height +Tide Direction	69386	0.973	8	-260864.4		
Tide Height +Tide Direction	69386	0.974	7	-260808.5	57.911	<0.001
Trevenon Bay						
Distance From Farm + Tide Height +Tide Direction	62201	0.972	8	-212421.7		
Tide Height +Tide Direction	62201	0.972	7	-212423.7	0.0483	0.826
Tide Height	62201	0.972	6	-212425.6	0.0748	0.784
Gorge Harbour						
Distance From Farm + Tide Height +Tide Direction	57500	0.947	8	-188294.4		
Tide Height +Tide Direction	57500	0.947	7	-188292.2	4.235	0.0396

Table 6: Fixed effects parameters and associated effect sizes (estimates are for the (ln+1) transformed chlorophyll-*a* concentration), standard error, df, t-values, and p-values for the best fit model for Westcott Bay.

	Value	Standard Error	*df*	t-value	p-value
Intercept	1.2973	0.07446	69360	17.4229	<0.001
Distance from Farm	0.0002073	0.00002639	69360	7.854	<0.001
Tide Height	-0.2686	0.03491	69360	-7.6922	<0.001
Tide Direction	-0.0264	0.01156	69360	-2.2823	0.0225

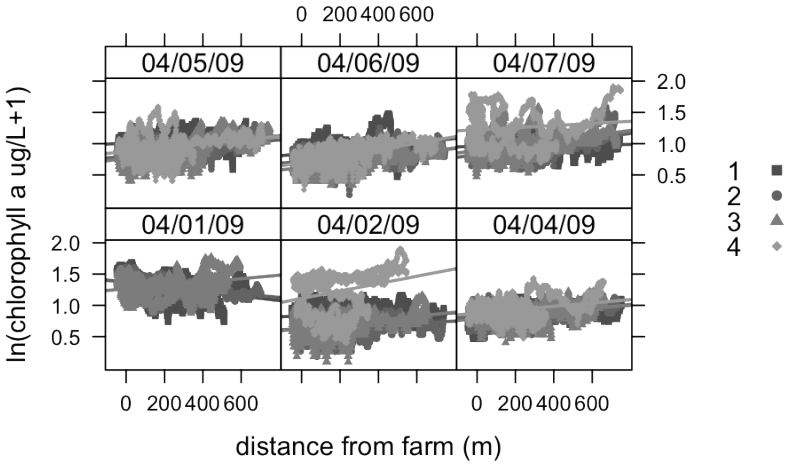

Figure 13: The relationship between distance from shellfish farm in meters and log + 1 transformed chlorophyll-*a* concentration (ln(chlorophyll-*a* ug/L + 1)). Each panel indicates a specific sampling day, each regression line within a panel indicates one transect (1-4), transect number represents successive transects completed on the same day.

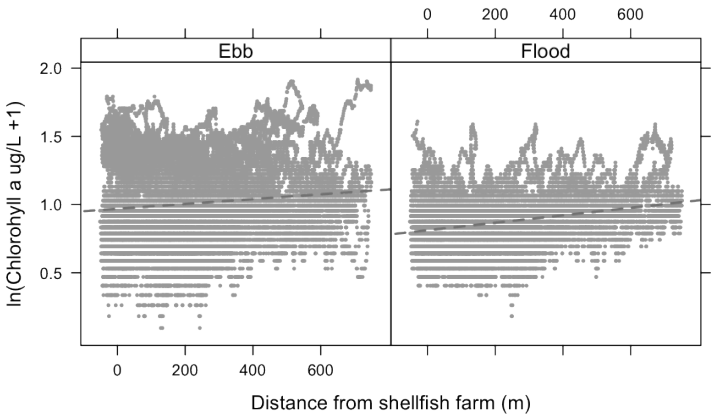

Figure 14: Log transformed chlorophyll-*a* concentration (ln(chlorophyll-*a* +1) vs. distance from a shellfish farm (m). Dashed line represents the linear model in ebb and flood tide.

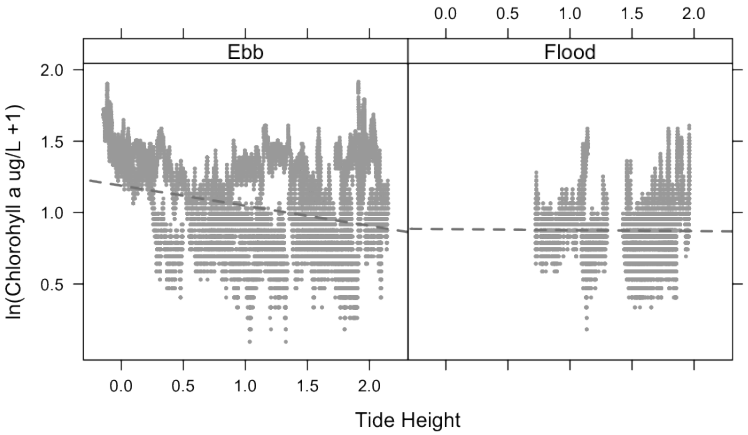

Figure 15: Log transformed chlorophyll-*a* concentration (ln(chlorophyll-*a* +1) vs. tide height. Dashed line represents the linear model.

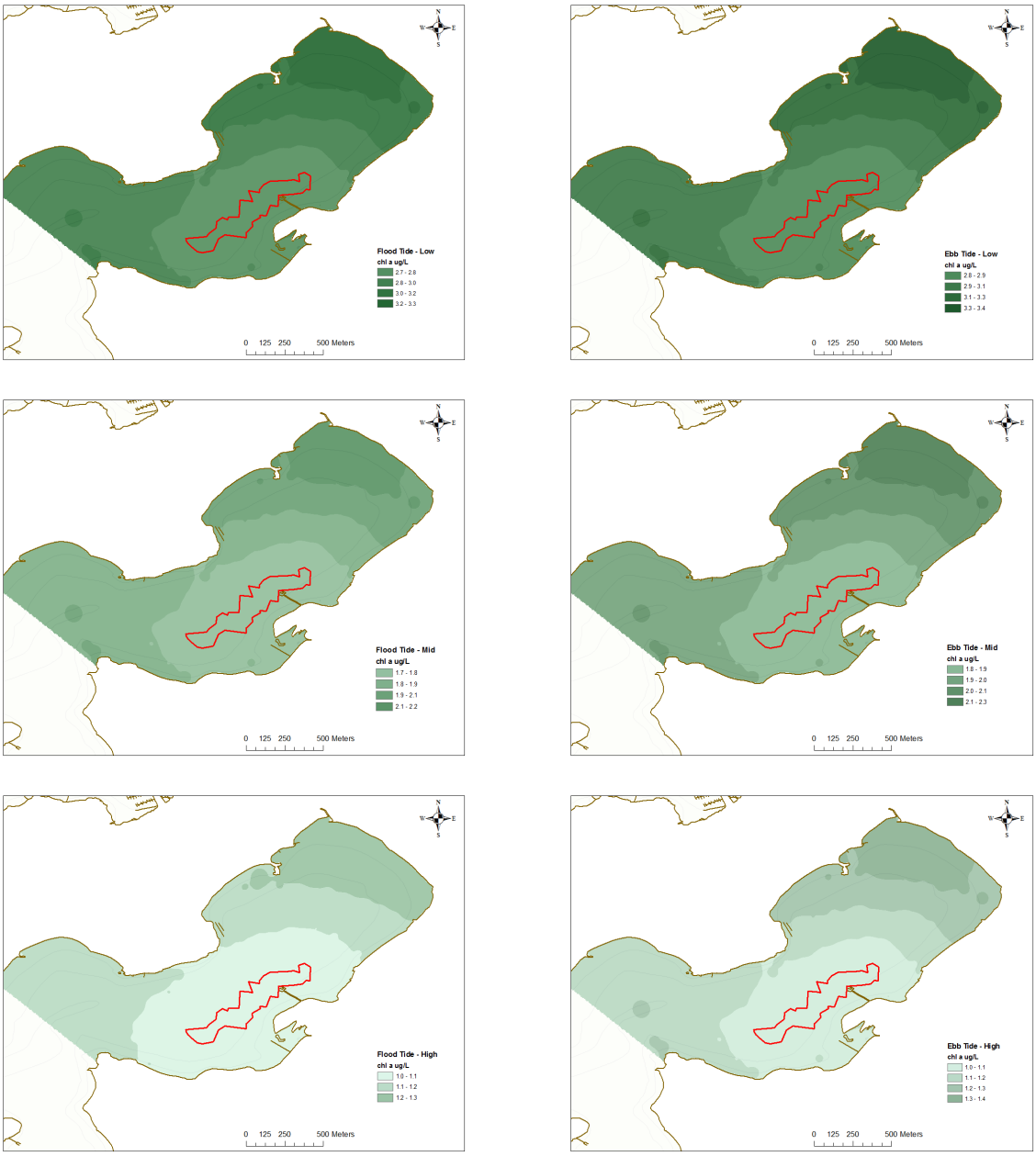

Figure 16: Maps showing the predicted chlorophyll-*a* concentration in Westcott Bay at three tidal heights (low tide, mid tide, and high tide) during an ebb and flood tide. The darker the green color, the higher the chlorophyll-*a* concentration. The red polygon marks the location of the shellfish farm.

2.3.1.2 Trevenon Bay

The best fit linear mixed effects model to describe chlorophyll-*a* concentration in Trevenon Bay included only one of the fixed effects parameters of interest: *tide height* (Table 5). The likelihood ratio test of the significance for the model fit showed no significant difference between successive model fits (L.Ratio=0.074836l, p-value=0.784, Table 5). This suggests that the most reduced model (lowest AIC) was the best fit for the data (Table 5). The correlation coefficient (phi) between successive measurements showed measurements were highly correlated (phi=0.972), and an (AR(1)) correlation structure in the model was needed. The parameter estimate of the fixed effect for the best fit model is shown in (Table 7), the estimate is for (ln+1) transformed chlorophyll-*a* concentration. Both *distance from farm* and *tide direction* were not significant parameters and were removed from the model (Table 5). When the data was pooled together a positive trend between chlorophyll-*a* concentration and tide height emerged (Figure 17).

Similar to Westcott Bay, it was hard to pull trends out of the raw data since parameters of interest could not be held constant during data collection (tide height and direction were changing over the course of a single transect). Therefore the maps provide a very useful tool to interpret the results of the model. The maps of predicted chlorophyll-*a* concentration in Trevenon Bay show the concentration of chlorophyll-*a* being the highest at high tide and decrease with tide height (Figure 18). Overall the model predicted a very small change in chlorophyll-*a* concentration in Trevenon Bay across different tidal heights (low tide 1.23 ug/L to high tide 1.99 ug/L (Δ =0.76 ug/L)).

Trevenon Bay was different from the other two bays studied because it had seven farm signals opposed to one point source farm signal. It was also impossible to navigate the data sonde through the farms as the density of culture was very high. Although a relationship between chlorophyll-a and proximity to any shellfish farm was not detected in Trevenon Bay, the density of culture (seven farms, effectively lining the entire foreshore), and geometry of the bay (long and narrow) made for an interesting new question: Is there an additive farm effect of distance from the mouth of the bay due to serial dilution caused by each successive farm? This changed the signal location from seven farm sites to a single axis perpendicular to the *mouth of the bay.* The results from

the mixed effects model including *mouth of the bay* as a fixed effect showed no significant effect on the fit of the model and was removed (p=0.186).

Table 7: Fixed effects parameters and associated effect sizes (estimates are for the (ln+1) transformed chlorophyll-*a* concentration), standard error, df, t-values, and p-values for the best fit model for Trevenon Bay.

	Value	Standard Error	df	t-value	p-value
Intercept	0.6439	0.1078	62178	5.9727	<0.0001
Tide Height	0.1043	0.02813	62178	3.7088	<0.0001

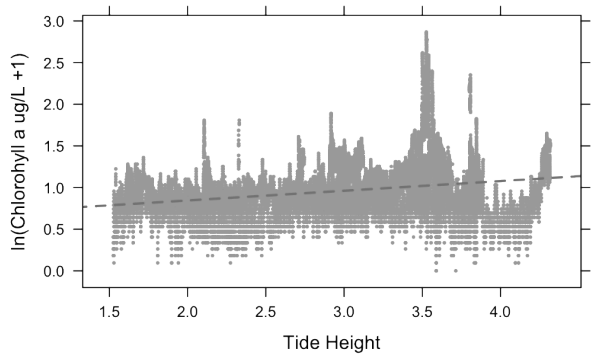

Figure 17: Log transformed chlorophyll-*a* concentration (ln(chlorophyll a +1) vs. tide height in Trevenon Bay. Dashed line represents the linear model.

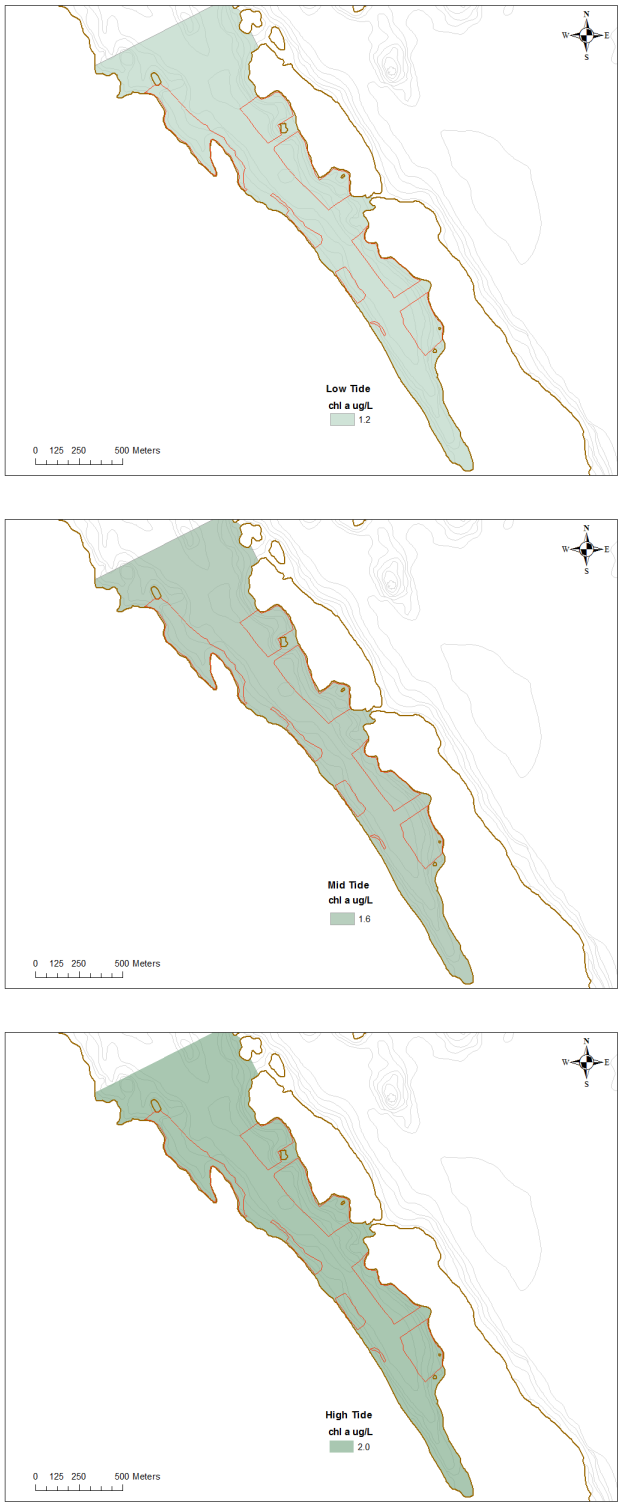

Figure 18: Maps showing the predicted chlorophyll-*a* concentration in Trevenon Bay at three tidal heights (low tide, mid tide, and high tide). The darker the green color, the higher the chlorophyll-*a* concentration. The red polygons mark the locations of the shellfish farms.

2.3.1.3 Gorge Harbour

The best fit linear mixed effects model to describe chlorophyll-*a* concentration in Gorge Harbour included all the fixed effects parameters of interest: *distance from farm*, *tide height*, and *tide direction* (Table 4). The likelihood ratio test to compare the two models showed that the best fit model was significantly better fit than the model that did not include the parameter *distance from farm* (L.Ratio=4.235, p-value=0.0396, Table 4). The correlation coefficient (phi) between successive measurements showed measurements were highly correlated (phi=0.947), and an AR(1) correlation structure in the model was needed. The parameter estimates of the fixed effects for the best fit model are given in (Table 8), estimates are reported for (ln+1) transformed chlorophyll-*a* concentrations. The relationship between distance from shellfish farm and chlorophyll-*a* concentration is shown in (Figure 19), each panel indicated a specific sampling day, each regression line within a panel indicates one transect (1-6), transect number represents successive transects completed on the same day. Most transects had a flat or slight positive slope with increasing distance from a shellfish farm and that six of the seven sample days the concentration of chlorophyll was very low (Figure 19). When the data were pooled together and split into two plots by tide direction (ebb, flood), an overall positive trend in the data with increased distance from farm was evident, but appeared to be exaggerated by sample day seven (Figure 20 (a)). When sample day seven was split away from the rest of the sample days the trend in the relationship between chlorophyll-*a* concentration and distance from the farm decreased for both ebb and flood tide (Figure 20 (b)). Pooled transacts from sample day seven however appeared to have a stronger positive trend that was more pronounced on an ebb tide (Figure 20 (c)). When the data were split into two plots by tide direction (ebb, flood), the overall trend with respect to tide height was positive, which was opposite to what the mixed effects model predicted. This trend line was influenced by the high chlorophyll-*a* concentrations from sample day seven (Figure 21 (a)). Again, when sample day seven was split away from the rest of the sample days the positive trend disappears and no relationship between chlorophyll-*a* concentration and tide height is observed for either ebb and flood tide (Figure 21 (b)). Sample day seven however appears to have a strong negative trend that is more pronounced on an ebb tide (Figure 21 (c)).

As shown in the other bays the maps provide a very useful tool to interpret the results of the model. The maps of predicted chlorophyll-*a* concentration in Gorge Harbour showed the concentration of chlorophyll-*a* being the highest at low tide with minimal differences between flood and ebb tide. The concentration of chlorophyll-*a* was slightly less on a flood tide than on an ebb tide. It was also obvious from the maps that tide height controlled the concentration of chlorophyll-*a* more than the effect seen from the presence of the shellfish farm. On a flood tide, the concentration of chlorophyll-*a* changed from 1.76 ug/L at high tide to 2.00 ug/L at low tide, whereas the maximum difference in chlorophyll-*a* observed across the bay on a flood tide was 0.11 ug/L (Figure 22). Overall the model predicted very small changes in chlorophyll-*a* concentration in Gorge harbour across different tidal heights and at different distances from the shellfish farm.

Table 8: Fixed effects parameters and associated effect sizes, standard error (estimates are for the (ln+1) transformed chlorophyll-*a* concentration), df, t-values, and p-values for the best fit model for Gorge Harbour.

	Value	Standard Error	*df*	t-value	p-value
Intercept	1.1513	0.1658	57462	6.9453	<0.001
Distance from Farm	0.0000604	0.00002934	57462	2.0599	0.0394
Tide Height	-0.02936	0.01337	57462	-2.1958	0.0281
Tide Direction	-0.04716	0.01448	57462	-3.2578	0.0011

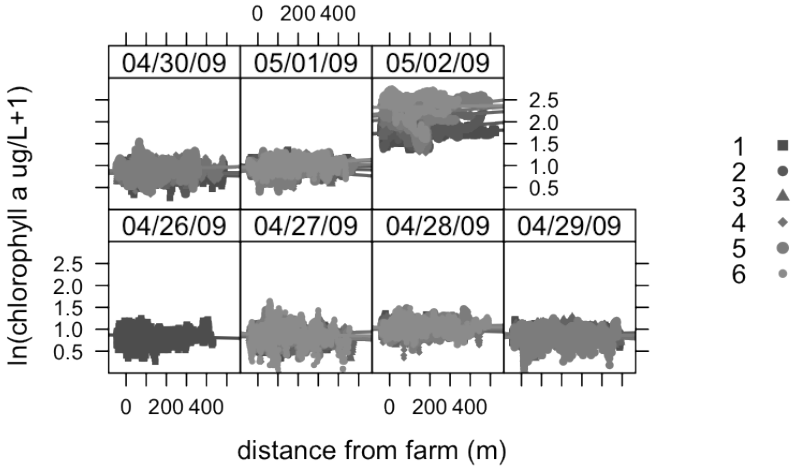

Figure 19: The relationship between distance from shellfish farm in meters and log + 1 transformed chlorophyll-*a* concentration (ln(chlorophyll-*a* ug/L + 1)). Each panel indicates a specific sampling day, each regression line within a panel indicates one transect (1-6), transect number represents successive transects completed on the same day.

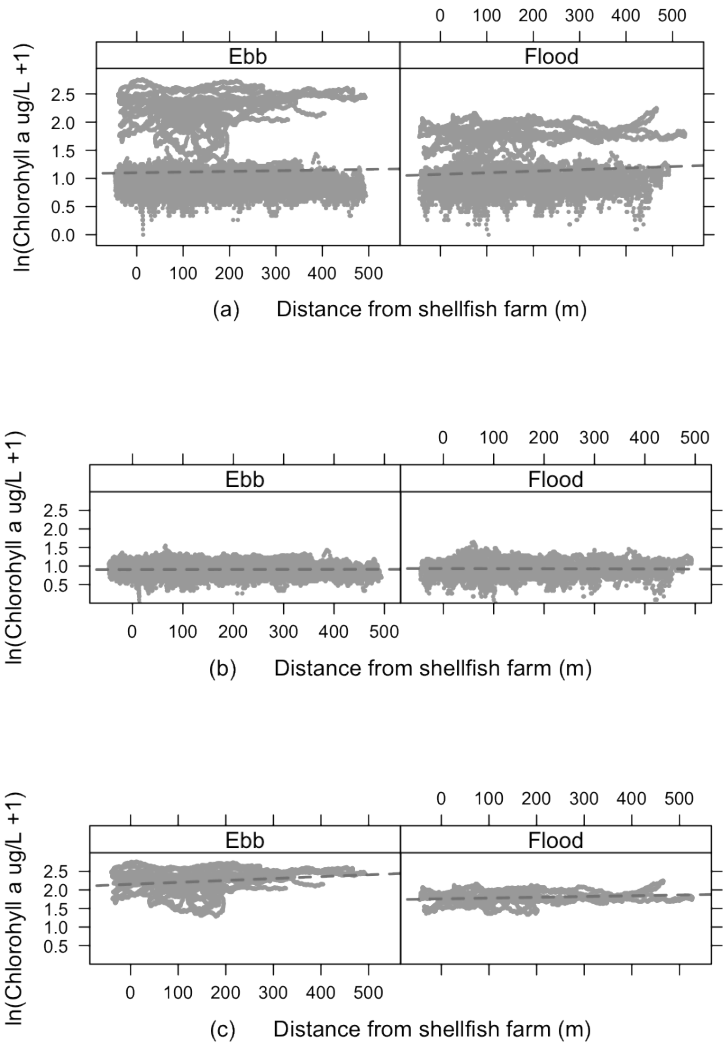

Figure 20: Log transformed chlorophyll-*a* concentration (ln(chlorophyll-*a* +1) vs. distance from a shellfish farm (m) in Gorge Harbour (a). Dashed line represents the linear model. Note: the data collected on day 6 is skewing the trend line for this site. Plots (b) (c) are the data from day 1-5, and day 6 respectively.

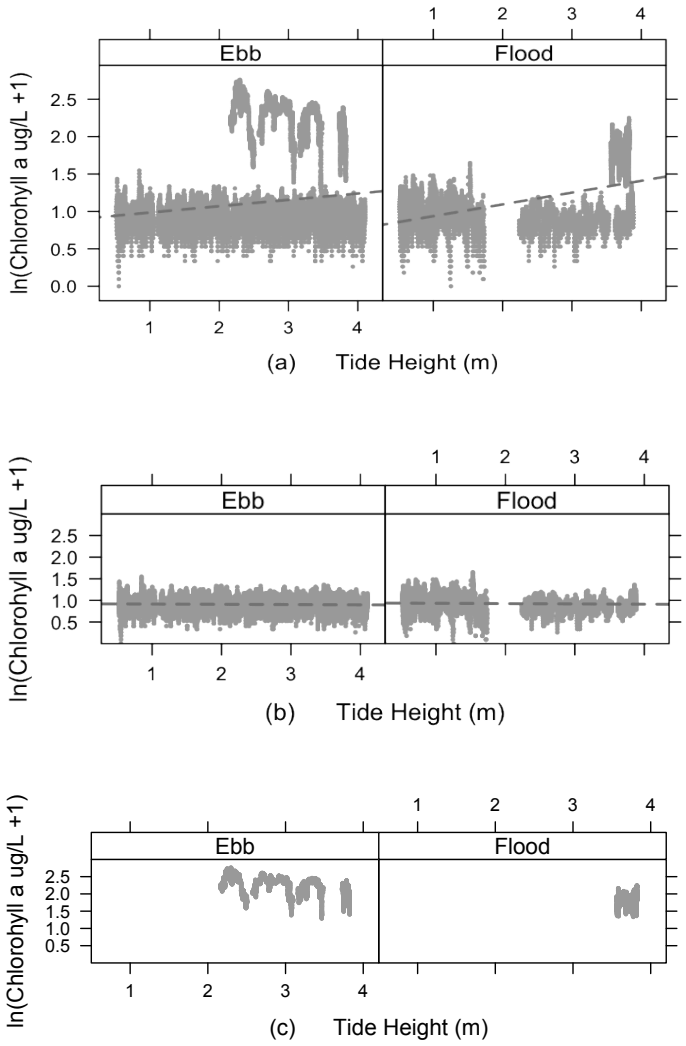

Figure 21: Log transformed chlorophyll-*a* concentration (ln(chlorophyll-*a* +1) vs. tide height (m) in Gorge Harbour (a). Dashed line represents the linear model. Note: the data collected on day 7 is skewing the trend line for this site. Plots (b) (c) are the data from day 1-6, and day 7 respectively.

Figure 22: Maps showing the predicted chlorophyll-*a* concentration in Gorge Harbour at three tidal heights (low tide, mid tide, and high tide) during an ebb and flood tide. The darker the green color, the higher the chlorophyll-*a* concentration. The red polygon marks the location of the shellfish farm.

3.4 Discussion

It was possible to detect a footprint of chlorophyll-*a* depletion around shellfish farms in some of the oceanographic environments sampled. Westcott Bay and Gorge Harbour had similar oceanographic characteristics, they both had a similar depth, a narrow opening at the mouth, and were relatively protected. The concentration of chlorophyll-*a* at both of these sites was influenced the most by tidal height. Westcott Bay showed the greatest overall total fluctuation in chlorophyll-*a* concentration. In the NE Pacific Ocean, during the spring and summer, temperature drives primary productivity in near-shore marine ecosystems (Ianson et al. 2001). Cold nutrient rich water is brought into the near-shore with every new tide, water is warmed in bays over the course of the tidal cycle, driving phytoplankton blooms (Ianson et al. 2001). This trend was reflected in the Westcott Bay and Gorge Harbour data. The highest chlorophyll-*a* concentration predicted by the models occurred in both bays on a low ebb tide, and the lowest predicted concentration occurred on a high flood tide. This indicated that tide drove the total chlorophyll-*a* concentration in each of the bays. New water was brought into the bay with a flood tide, the ambient chlorophyll-*a* concentration in the bay was diluted and the lowest concentration of chlorophyll-*a* was observed at the high flood tide. As the direction of the tide switched and started to ebb out of the bay, the chlorophyll-*a* concentration began to increase, and the maximum predicted chlorophyll-*a* concentration was observed on the low ebb tide.

Although there are many factors that control the total phytoplankton abundance in an ecosystem, the three main factors required for phytoplankton to bloom are: nutrient availability, sunlight, and temperature (Ianson et al. 2001). The carrying capacity of a given ecosystem is dictated by its primary production. Organisms within an ecosystem utilize this primary productivity and energy is transferred up the food chain. The introduction of shellfish aquaculture to an ecosystem introduces a new phytoplankton sink in that ecosystem. If the resources in a particular environment are limiting, the allocation of those resources are re-assigned to feed the farmed animals.

A second trend common to both Westcott Bay and Gorge Harbour was the presence of a depletion footprint around the perimeter of the farm. The magnitude of change across both bays was strongest when the concentration of chlorophyll-*a* was

highest (low ebb tide). This indicated the longer the resident time of water spent in either bay (increased water residency time) the greater the impact foraging farm animals had on phytoplankton abundance. Although Westcott Bay and Gorge Harbour experienced similar trends with respect to the impact of tide height, tide direction, and presence of a shellfish farm, Gorge Harbour was drastically influenced by one sampling day – sample day seven. When the total ambient chlorophyll-*a* concentration was low the effect of tide height, tide direction, and proximity to a shellfish farm was much more difficult to observe, as the total observable change from each of these factors was low. Interestingly, when the magnitude of change due to tide was compared with the magnitude change due to proximity to the shellfish farm for the two sites, the ratio of difference (Δ chl-*a* – tide height/ (Δ chl-*a* – shellfish farm) was approximately the same (Westcott Bay 0.66/1.67= 0.39; Gorge Harbour 0.11/0.29=0.38). Other studies that have documented declines in chlorophyll-*a* in proximity to shellfish farms were primarily observing the declines due to mussel farms (Lloyd 2003, Grant et al. 2008). Mussels are also bivalve suspension feeders that feed on ambient resources from in the water column.

The results from Trevenon Bay suggest that chlorophyll-*a* concentration is controlled primarily by tide height, and that tide direction and distance to a shellfish farm do not play a significant role. Trevenon Bay was different oceanographically from the other two study sites. The bay is very deep ~30 meters in the center and had little protection from wind and waves, with the widest location in the bay occurring at the mouth. Trevenon Bay was approximately 3 km long and had extensive culture along all of its shore line. Due to these differences in physical characteristics (exposure, and depth) the processes that control phytoplankton abundance in this bay were likely quite different than the processes that control phytoplankton in sheltered shallow bays. When nutrient rich water entered Trevenon Bay on a flood tide, the proportional depth of the water did not change dramatically, thus inhibiting rapid warming of the water column and phytoplankton growth typical of the other two bays. Also, since the mouth of Trevenon Bay is very wide, water is unlikely to be trapped in the bay longer than one tidal cycle, therefore turnover is relatively rapid. It is possible that due to the sampling methodology, it was not possible to detect differences occurring at this site that may have been present. Sampling was only able to occur in the center of Trevenon Bay where no farms were

present. Because the orientation of the bay was straight and narrow, sampling occurred at the deepest and most exposed section of the bay. Therefore similarities that may exist between Trevenon Bay and the other sites were not accessible, such as local changes in chlorophyll-*a* concentration within farm boundaries. Since Trevenon Bay offered a more complex study site compared to the other two bays studied, it was possible to explore the data in a slightly different way. Instead of using each farm as it's own point source of depletion, the relationship of chlorophyll-*a* concentration from mouth to head was also investigated. The distance between each data point and the mouth of the bay was calculated to determine if there was a serial dilution effect from having successive farms in the same bay. The results showed no significant difference in chlorophyll-*a* concentration between the mouth and head of the bay, further indicating that the farms were not significantly depleting chlorophyll-*a* in Trevenon Bay.

Chapter 4

4.0 General Discussion

4.1 Overview of Results

The global population continues to grow at an exponential rate and it is evident that there is a need to maintain a continuous global food supply. Food from aquatic systems continues to be dominated by capture fisheries. The aquaculture industry has also continued to grow, and in 2008 it accounted for 46% of the global food fish supply (FAO 2010). Marine aquaculture has emerged as an important food source, although the majority of aquaculture occurs inland. In British Columbia, aquaculture is primarily marine, with salmon and shellfish accounting for the majority of species cultured. Shellfish aquaculture accounts for significantly less production and value compared to salmon aquaculture, although the amount of foreshore dedicated to farming shellfish is nearly half (44%) the total area utilized by all aquaculture in the Province (MOE 2010). Shellfish aquaculture in British Columbia is dominated by introduced Pacific oysters (74%) (MOE 2010) which are known to be very efficient generalist filter feeders that can grow faster and larger than native species (Ruesink et al. 2005, White et al. 2009). Farmed Pacific oysters could be influencing the carrying capacity of inshore marine systems because shellfish aquaculture is an extensive form of aquaculture in which farmed animals feed exclusively on naturally occurring food and have the ability to deplete planktonic material in the surrounding water column (Little and Bunting 2005).

Water column chlorophyll-*a* concentration and the change in growth of Pacific oysters was explored over two field seasons to obtain a better understanding of the relationship between Pacific oysters and phytoplankton (water column chlorophyll-*a*) in the near shore NE Pacific Ocean. In Chapter 2, two bays were compared, one exposed to shellfish aquaculture (Westcott Bay) and one non-exposed reference bay (Fisherman Bay). The concentration of chlorophyll-*a* was compared at three locations within each bay at two depths, and at high and low tide. The results indicated that position in the bay

did not predict chlorophyll-*a* concentration at either site. The chlorophyll-*a* concentration was found to be significantly lower at the surface compared to at depth in Westcott Bay, whereas in Fisherman Bay the chlorophyll-*a* concentration was found to be significantly lower at high tide compared to low tide. Although different parameters were found to be significant in each of the bays, the same trend was seen in all three parameters of interest at both sites (chlorophyll-*a* concentration was lower at the surface and at high tide, with no trend observed at different sample positions in either bay). Although position in the bay did not predict chlorophyll-*a* concentration at either site, the results from the oyster growth experiment that compared the size of 100 Pacific oysters at two locations within Westcott Bay Seafarm, showed oysters were significantly smaller when grown in the center of the farm compared to oysters grown at the farm's edge. These data suggested that although differences in water column chlorophyll-*a* concentration were not observed using vertical point sampling, phytoplankton within the farms perimeter may be limiting and that competition between farmed Pacific oysters for common food resources may be present in Westcott Bay. In addition, due to the structure of the farm below the surface of the water, water may be trapped within the farms perimeter longer then water outside of the farm, further limiting food supply to feeding farmed Pacific oysters. These results suggest that a depletion signal may be present in Westcott bay that vertical point sampling was not detecting.

Chapter 3 explored the horizontal spatial relationship between chlorophyll-*a* concentration and proximity to a shellfish farm, to further investigate the relationship between water column chlorophyll-*a* concentration and proximity to farmed Pacific oysters. Three bays with varying amounts of shellfish culture were horizontally sampled for a period of six days each. Two of the sample bays, Westcott Bay and Gorge Harbour, had similar physical characteristics in terms of exposure, and depth, whereas Trevenon Bay, the other sample bay, had very different physical characteristics. Both Westcott Bay and Gorge Harbour (referring to the area sampled in Gorge Harbour) were sheltered shallow bays, with only one shellfish farm. Trevenon Bay was an exposed deep bay, with seven shellfish farms. The results from the horizontal spatial sampling showed a footprint of chlorophyll-*a* concentration depletion in proximity to a shellfish farm in both Westcott Bay and Gorge Harbour, the intensity of which changed at different tidal

heights on both flood and ebb tides. Similar to the results from Chapter 2, tide height also played a significant role in chlorophyll-*a* concentration in sheltered shallow bays. The same trend in tide height observed in Chapter 2, which showed that the chlorophyll-*a* concentration was lower at high tide versus low tide, was also observed in the horizontal spatial sampling in Westcott Bay and Gorge Harbour (sheltered shallow bays). The data from Trevenon bay did not show a relationship between chlorophyll-*a* concentration and proximity to a shellfish farm. As well, there were no differences in chlorophyll-a concentration at flood and ebb tide. Chlorophyll-*a* concentration was also investigated mouth to head of the bay to test for serial dilution from successive farms. No relationship was observed from mouth to head. However, the results did indicate that chlorophyll-*a* concentration was significantly higher at high tide versus low tide, although the magnitude of change observed across the entire bay was very low.

Although many similar trends were observed between the two field seasons (Chapter 2 and Chapter 3), the main difference was the detection of a small chlorophyll-*a* depletion footprint in proximity to a shellfish farm in Westcott Bay and Gorge Harbour during field season two. The methodologies used in the two field seasons were very different. In field season one, vertical point sampling was employed to measure changes in chlorophyll-*a* concentration at different locations in the bay, at two depths and two tidal heights. Using this methodology, the exact chlorophyll-*a* concentration was extracted from parcels of water and compared. This was labour intensive and allowed for only 12 water column measurements to be made per day (six per tide height, and six per depth at each of the three samples sites). In field season two, the relative concentration of chlorophyll-*a* was determined using a fluorometer allowing for a measurement to be made every two seconds, totalling approximately 10,000 water column measurements per day. Phytoplankton are known to be very "sticky" causing them to aggregate together and form layers in the water column (Kiorboe and Hansen 1993). Due to the limited sample size in field season one, it was possible that natural variability in phytoplankton assemblages within the water column caused the signal from the farm to be undetectable. The results from field season two show that tide height controls chlorophyll-*a* concentration more than proximity to a shellfish farm, further indicating the possibility that differences in chlorophyll-*a* concentration caused by both tide height and depth

masked the point source depletion signal (farm signal) from the shellfish farm in field season one.

4.2 Methodological Strengths and Weaknesses

Two methods for quantifying chlorophyll-*a* concentration were employed during this research. Each method had inherit strengths and weaknesses associated with its use. In Chapter 2, extraction was used to compare differences in chlorophyll-*a* concentration at three different locations in two bays, Westcott Bay and Fisherman Bay, at two depths and at two tidal heights. The extraction method is the only way to determine the exact amount of chlorophyll-*a* in a given parcel of water. Chlorophyll-*a* is the main pigment in plants that converts light energy into chemical energy during photosynthesis (Hoepffner and Sathyendranath 1991). The extraction method takes a subsample of sea water that contains planktonic cells and filters it through a GF/F glass microfiber filter paper. This process collects planktonic material on the filter paper, the chlorophyll-*a* can then be extracted from the planktonic cells using a solvent (90% acetone). The extracted chlorophyll-*a* can then be measured using a fluorometer. This methodology provides the best absolute measure of chlorophyll-*a* concentration in a given parcel of water, but does not allow for many samples to be taken, as extracting is time consuming and expensive. In Chapter 3 *in-situ* florescence was used to compare relative differences in chlorophyll-*a* concentration across three different bays. Using this methodology, it was possible to collect a large number of water column chlorophyll-*a* measurements in a short period of time. Instead of collecting water samples and extracting the chlorophyll-*a* concentration directly from the planktonic cells, this method used a sensor to estimate chlorophyll-*a* concentration by instantaneously detecting algal fluorescence. The sensor emitted a blue light (centered around 470 nm) and measured the fluorescence of all species in the water column. Fluorescence from chlorophyll-*a* in phytoplankton was reflected back at the chlorophyll-*a* sensor and was recorded. This methodology has been shown to provide a relative estimate of chlorophyll-*a* concentration for a large number of samples in the water column, but not as accurate at measuring exact concentrations, as were provided by the extraction method. This is primarily because different species of phytoplankton

fluoresce differently under different light conditions, temperatures, and different times of the day (Lorenzen 1966, Sackmann et al. 2008).

In Chapter 3, the spatial relationship between water column chlorophyll-*a* concentration and proximity to a shellfish farm was explored. Three different bays were studied to determine if similarities between areas with different physical and biological characteristics could be observed. Studying three different areas created difficultly in maintaining the same sampling methodology at each site. The seafarm in Westcott Bay suspended animals from floating buoys that were spaced apart in a way that was easy to navigate within the farms boundaries. The Gorge Harbour seafarm suspended animals from floating rafts, which were also arranged in such a way it was possible to navigate within the farms boundaries. However, because rafts were used instead of individual buoys, it was only possible to navigate one route within the farms boundary. The seafarms located in Trevenon Bay suspended animals from both buoys and rafts, but arranged the area within each farm so densely it was impossible to sample within the farms boundaries. Since it was impossible to sample within the farm boundaries in Trevenon Bay, it is important to consider that limitations in the sampling methodology missed data from this site.

4.3 Other Potential Sources of Error

In nature it is impossible to maintain the same type of controlled environment as used when conducting research in the laboratory setting. The physical elements that could not be controlled included: strength of the tide, bathymetry (depth), nutrient load, local weather conditions (such as air temperature, duration and intensity of sunlight, and wind), local currents, and physical structures in the body of water (such as moored boats, buoys, docks and other users of the area). The biological elements that could not be controlled included: species of phytoplankton, size of shellfish farm, and the age, species, density, and filtering capacity of farmed and native suspension feeding animals within the bay (inter/intraspecific species competition). These elements create background noise in the data and make it more difficult to discern specific trends of interest. Given that this system had significant "noise" that could create variation and hide specific trends of

interest, field season two employed an intensive sampling design in an attempt hyper-sample each bay.

4.4 Summary and Overall Conclusions

Suspension feeding bivalves are known to cause depletion effects of water column chlorophyll-*a* concentration in other parts of the world (Lloyd 2003, Grant et al. 2007, Grant et al. 2008). The majority of research comparing water column seston in proximity to cultured bivalves, uses mussels as the primary species of interest. Mussel farming is employed in much the same way as Pacific oyster farming, where mussel farms are extensive and farmed animals feed off ambient material in the water column. The results from field season two suggest that Pacific oyster farming can also cause measureable depletion of water column chlorophyll-*a* concentration in near-shore sheltered bays, as shown in Westcott Bay and Gorge Harbour, similar to mussel farming. The trend shown in Trevenon Bay suggests that some areas along the NE Pacific coast maybe unaffected by shellfish culture. Although the ambient chlorophyll-*a* concentration in Trevenon Bay was very low during the sampling period, a map showing telemetry locations from all seabirds, suggests that the area just north of Trevenon Bay can be very productive (Figure 23). Perhaps, physical differences in Trevenon Bay and naturally high productivity (inferred from biological indicators) in the area, extensive aquaculture could be possible without a measureable depletion of chlorophyll-*a* concentration.

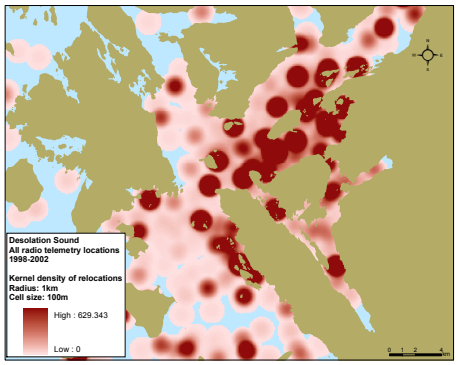

Figure 23: Telemetry locations of all birds in Desolation Sound between 1998-2002 (Data obtained by the Simon Fraser University Marbled Murrelet Research Group, 1998-2002, under the direction of Fred Cooke and David Lank, analyzed by Jennifer Barrett).

The NE Pacific coast primarily farms Pacific oysters, it is possible that in addition to changes in the total abundance of phytoplankton indirect changes in the species composition of phytoplankton adjacent to farms may also occur (although this was not tested during the research). The Pacific oyster is an introduced, generalist, marine bivalve to the Pacific coast of North America. As a generalist it has the ability to ingest a wide range of particle sizes compared to native species which tend to be more specialized in their feeding preference (Quayle 1988), and over time has the potential to outcompete native species for common food resources (Ehrlich 1986, Ruesink et al. 2005). Local differences in oyster growth suggest that phytoplankton within the farms perimeter was limiting and that intra-specific competition between farmed Pacific oysters for common food resources was higher in the center of the bay where the density of feeding oysters was highest. Oysters grown in the center of the bay were surrounded on all sides by other feeding oysters, whereas oysters grown at the periphery of the farm experience have less competition for common resources. This enabled oysters on the periphery to access more food to grow larger than oyster grown in the center of the farm. Overall, this research demonstrated a measureable gradient of phytoplankton around shellfish farms in sheltered shallow bays with depletion closest to the farm site, as well as, greater oyster growth at the periphery of shellfish farms where phytoplankton would be predictably in greater abundance. These data are important indicators suggesting that shellfish farms along the west coast of Canada and the United States have potential to cause depletion of phytoplankton that may cause cascading effects through the ecosystem.

4.5 Future Directions

This research demonstrated that it was possible to detect a point source depletion effect around a shellfish farm in sheltered shallow bays in the early spring. It was also demonstrated that local differences in food availability may exist that influence the growth of farmed oysters within a farms perimeter. Through these findings, new questions about the relationship between water column chlorophyll-*a* concentration and shellfish farms emerged. (1) Are Pacific oysters preferentially selecting phytoplankton species that have higher nutritional value? (1a) If so, does this further compound the

detected chlorophyll-*a* gradient observed? (2) Does the strength of the point source depletion signal (farm signal) change with season? (3) When do the local differences in Pacific oyster growth emerge over the course of the year (i.e. In what season (time of year) is intra-specific competition for food the highest within the farm boundaries?)? (4) Do shellfish farms influence bay wide biodiversity? (5) What is the cumulative effect of multiple shellfish farms within in a bay/inlet/region/coastline? These questions could further extend the state of knowledge in this area and address the cost benefit relationship of shellfish culture in the near shore NE Pacific Ocean.

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