Salmon farm wastes as a potential nutrient subsidy to adjacent intertidal communities in Clayoquot Sound, British Columbia.

By

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Abstract

Intertidal community structure, species biomass and stable isotope (^{15}N) composition was investigated in Clayoquot Sound, British Columbia adjacent to five open net-pen salmon farms and four reference locations. Open net-pen salmon aquaculture releases organic and inorganic wastes to the local environment, providing a nutrient subsidy to many marine organisms. The availability of these wastes to adjacent intertidal communities is unknown and variation in resource availability to the intertidal community is emerging as a topic of interest. Using a three-level, spatially nested sampling design, I examined intertidal invertebrate community structure and biomass of intertidal mussels, *Fucus* and phytoplankton, species known to use salmon farm wastes . The stable isotope (^{15}N) content of eelgrass, *Fucus* and mussels was used as a tracer of salmon farm derived nutrients into adjacent intertidal communities. No significant effect of farm waste on biomass of phytoplankton, *Fucus* or mussels was detected, however *Fucus* biomass was noted to be inversely proportional to distance at two farms, and a reference location that had been a farm previously for several years. Salmon feed and muscle tissue were significantly more enriched for ^{15}N than eelgrass, *Fucus* and mussels. On average, eelgrass, *Fucus* and mussels were not significantly different from one another, occupying the same range of 6-10 ‰ ^{15}N. No effect of distance of farm was found for eelgrass or *Fucus*. Mussels collected directly from farm net cages showed no enrichment in comparison to mussels collected from pristine areas. Farms had no effect on community composition, species richness, predator and total species abundance on large (kms) or small (100's of meters) scales. Species richness and predator abundance were positively correlated with variation in *Fucus* biomass in accordance with current species richness-productivity theories, and trophic-dynamic models. Most of the variation for all variables was explained by small (meters) or large (kilometers) scale variation, whereas mesoscale (100's of meters), and treatment (farm/no farm) explained little. Likely reasons for no obvious effect of farms on intertidal communities are the distances of farms from the intertidal zone, flushing rate by currents, pulsed release of wastes from farms and natural variation.

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Chapter 1.

Are salmon farms a source of nutrients to adjacent intertidal communities in Clayoquot Sound, British Columbia?

A community is an example of an ecological pattern, a collection of organisms connected through spatial proximity, species interactions (directly and indirectly), abiotic processes and evolutionary constraints. Community structure is the composition, distribution and abundance of the species composing the community. Community structure is shaped by a complex and dynamic combination of processes such as resource availability (Wootton and Power 1993), primary production (Bustamante *et al.* 1995*a,b*, Menge *et al.* 1997*a*), physical factors (Dayton 1971, Connell 1961), and species interactions (Hairston *et al.* 1960, Menge and Sutherland 1976, Nielson 2000). Each of these processes operate on a range of spatial and temporal scales.

Studies of community structure in the marine intertidal zone began with the roles of competition and predation (Connell 1961, Paine 1974, Menge and Sutherland 1976, Lubchenco 1978, Menge 1978) and physical factors (Connell 1961, Dayton 1971). The ocean was considered to be an unlimited source of nutrients available to intertidal organisms, and was thought not to vary appreciably over the small spatial scales covered by these studies (Menge 1991, 1992). Although executing controlled experiments involving the manipulation of nutrients in an open, dynamic system such as the ocean is logistically challenging (Menge *et al.* 1997a, Bustamante *et al.* 1995*a,b*), both natural and manipulative studies conducted on a range of spatial scales that investigate the roles of nutrients and primary productivity in structuring intertidal communities have emerged (Duggins *et al.* 1989, Bustamante *et al.* 1995, Menge *et al.* 1997)

Nutrient subsidies and natural variation in phytoplankton biomass have variable direct effects on intertidal organisms, and indirect secondary effects on their communities. Nutrient subsidies in the form of detrital kelp and seabird wastes affect the growth, abundance and distribution of intertidal organisms. Translocation experiments with mussels (*Mytilus edulis*) and barnacles (*Balanus glandula*) between Aleutian Islands with and without kelp resulted in growth rates of these species to be increased by 2-5x at islands with kelp (Duggins *et al.* 1989). Stable carbon isotope analysis confirmed a large contribution by detrital kelp as a food source for suspension feeders. Exclusion of detrital kelp from intertidal limpets' (genus *Patella*) diet around the coast of South Africa caused significant mortality and decreased body mass (Bustamante *et al.* 1995*a* and *b*). Detritus from senescing subtidal kelp subsidizes the diet of limpets in areas of high kelp abundance allowing them to maintain densities nearing saturation of available substrate. These limpets exert strong top-down control over attached algal growth and diversity, and reduce space occupancy by filter feeders (Bustamante *et al.* 1995*b*).

Seabird wastes are a nutrient subsidy to aquatic vegetation. Production and community structure of seagrass beds were examined in Florida at islands with and without seabird colonies (Powell *et al.* 1991). Seagrass standing crop and species richness were elevated at islands with bird colonies, and the dominant seagrass species differed between the two island treatments. Increasing bird derived subsidies by installing perches produced changes in islands without colonies that were similar to islands with bird colonies. Wootton (1991) saw differences in distribution, abundance and dominance of algal and lichen species in the high intertidal zone with and without seabird colonies. Four of the 18 species investigated benefited positively from a subsidy of bird derived nutrients, while 14 other species were negatively affected directly or indirectly.

Experimental (nutrient infused clay pots) and natural (*El Nino*/non *El Nino* years) variation in nutrient concentrations were used to link variation in ecosystem processes (nutrients and productivity) to community dynamics (Wootton *et al.* 1996). Nutrients produced variable results on community dynamics while maintaining food chain stability, and top-down grazing effects were typically stronger than bottom-up nutrient effects. However, the temporal and spatial scale of this study restricted the formation of complete, stable intertidal communities to form on clay pots, thus limiting the conclusions. Menge *et al.* (1997) investigated two distinctly different communities (algae vs. invertebrate dominated) 80km apart along the coast of Oregon for differences in nutrients and nearshore phytoplankton biomass as an explanation. Nutrients varied inconsistently and were never scarce enough to limit phytoplankton growth. Phytoplankton biomass and organic particulates were always higher at the filter feeder dominated community. Around the coast of South Africa, a clear east to west gradient of increasing nutrients, primary production, and biomass of grazers was found (Bustamante *et al.* 1995*a*). Dominance patterns of algal species also changed with the gradient. Although the number of studies focused on the effects of variation in nutrient availability and primary production on marine intertidal community structure are few, the evidence they provide is compelling as it spans several spatial scales and different focal species.

Salmon net-cage farming may provide a forum for further investigation. In British Columbia (BC), salmon net-cage operations practice external waste management. These wastes are food and fertilizer for marine animals and plants and there is evidence that they enter the food chain (Williams and Ruckelshaus 1993, Ahn *et al.* 1998, Vizzini and Mazzola 2004). Consistently high volumes of waste output, static position, and clustered arrangement (in areas of BC such as the Broughton Archipelago and Clayoquot Sound), make large scale (kilometers), replicated studies possible. Smaller scale community variation can be linked to larger scale ecological processes when multiple spatial scales are investigated simultaneously. The residency of a salmon farm in a single location over years allows for the investigation of changes in population dynamics and lagged responses over time.

Over a cultivation cycle, 67% of feed (as dry pellets) added to open net pens is converted to salmon biomass (1.5 feed conversion ratio, Naylor *et al.* 1998), the balance is released directly to the ocean as solid and particulate waste, primarily in the form of salmon metabolic wastes and excess feed. Fish, suspension and deposit feeders consume the particulate portion of fish farm wastes (Widdows *et al.* 1979, Wallace 1980, Jones and Iwama 1991, Troell *et al.* 1999); plants, macroalgae and phytoplankton utilize dissolved nutrients (Valiela *et al.* 1992, Williams and Ruckelshaus 1993, Ahn *et al.* 1998). During this study (2001-2003), BC had 80-83 operational farms and an average annual production of 67 700 tonnes (range 50 000-85 400 tonnes round wt) (BC Salmon Farmers Association, http://www.salmonfarmers.org), which averages 816 tonnes fish per farm per year, and 270 tonnes of waste per farm per year. It is estimated that 70% of nitrogen (~50kg per 1000kg feed) and 80% of phosphorus (~8 kg per 1000kg feed) added as feed during a production cycle are released to the environment (Holby and Hall 1991, Hall *et al.* 1992).

Integrating the culture of suspension feeders (oysters or mussels) and macroalgae with salmon has been examined as a candidate solution to reducing the amount of dissolved and particulate wastes released to the surrounding environment with variable results (Wallace 1980, Jones and Iwama 1991, Stirling and Okumus 1995, Taylor *et al.* 1995, Ahn 1998, Troell and Nordberg 1998, Troell *et al.* 1999*a,b*). Mussels (*Mytilus edulis*) growing on salmon farms were found to grow twice as fast as mussels growing subtidally and intertidally nearby (Wallace 1980). This result was attributed to a farm derived resource subsidy in the winter when phytoplankton densities are low. Concentrations of chlorophyll *a* and particulate organic matter (POM), and growth of mussels were higher when growing near salmon farms in Scotland than at mussel farms (Stirling and Okumus 1995). Increased growth rate of mussels was attributed to a year round subsidy of POM by salmon farms. Jones and Iwama (1991) varied the distance of hanging baskets filled with oysters from a salmon farm and found oyster growth to follow a chlorophyll *a* and POM gradient which peaked at the farm. Kelps (*Laminaria* *saccharina* and *Nereocystis luetkeana*) are efficient at removing dissolved nitrogenous wastes from salmon farms, showing linear increase of nutrient uptake with increasing concentrations (Ahn *et al.* 1998). In contrast, Taylor *et al.* (1995) found no effect of salmon farm wastes on mussel growth (condition index, crude protein and carbohydrate content), POM or chlorophyll *a* concentration. However, they attribute these observations to dense populations of mussels growing on the farm removing any farm-derived POM or phytoplankton. Salmon farms were found to have no effect on the growth of cultured mussels in Tasmania, when compared to those cultured distant from the farm (Cheshuk *et al.* 2003). They attribute these observations to a combination of ambient concentrations of phytoplankton and POM always exceeding the filtration capacity of mussels, mussels were cultured too far from the farm, and dilution of salmon farm derived POM by flushing.

Although these studies do not discuss their results in the context of the ecological community, they do provide important information on how individual species respond to nutrient subsidies supplied by salmon farms, giving insight into how these species might respond and affect their communities under similar conditions.

A drawback to the previous studies is correlational evidence. There are no conclusive indicators that salmon farm derived nutrients are assimilated into the local food web. Here I introduce the heavy stable isotope of nitrogen (^{15}N) to serve this purpose. N^{15} is preferentially retained in an organism during metabolic reactions over the lighter and more common isotope ^{14}N, and bioaccumulates on average 3-5$^0/_{00}$ per trophic level (Minagawa and Wada 1984, Peterson and Fry 1987). The ratio of $^{14}N/^{15}N$ can be measured with high precision using mass spectrometry (Peterson and Fry 1987). Many studies have used stable isotopes to act as natural tracers of nutrient flow between adjacent ecosystems (Kline *et al.* 1990, 1993, Reimchen *et al.* 2003), to indicate trophic position of organisms (Welch and Parsons 1993, Cabana and Rasmussen 1996, Vander Zanden *et al.* 1999), and trace the movements of animals (Hansson *et al.* 1997). Cultured salmon, the species composing their feed and subsequently their wastes occupy a higher trophic position than either the primary producers and mussels investigated in this study or their resources (Minagawa and Wada 1984, Welch and Parsons 1993). Therefore, intertidal mussels and primary producers utilizing salmon farm derived nitrogen should appear to be at an elevated trophic position relative to those without access.

The purpose of this study was to quantify changes (if any) in intertidal community structure that can be linked to nutrient subsidy by salmon farms. In order to provide evidence for this linkage, I measured biomass and/or stable isotope composition in nearshore phytoplankton, the intertidal brown algae *Fucus distichus,* the eelgrass *Zostera marina,* and the mussel *Mytilus edulis.* These species were chosen based on the assumptions of direct usage of salmon farm derived wastes and thus produce the most immediate and detectable responses. The specific predictions of this study were threefold:

1) the presence of a salmon farm would result in increased biomass of intertidal phytoplankton, *Fucus* and mussels, which would be detectable as a gradient; the magnitude of response inversely proportional to distance from the farm.

2) organisms assimilating salmon farm derived nutrients would reflect this subsidy as being enriched for ^{15}N relative to those without access. This response would also increase in magnitude with decreasing distance from farms.

3) invertebrate community structure could reflect this nutrient subsidy as increased abundance of individuals, addition of species, or addition of trophic levels, measured as significantly dissimilar from areas without access to this subsidy.

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Chapter 2

Comparison of biomass of phytoplankton, the brown algae *Fucus distichus*, and the blue mussel *Mytilus edulis* in intertidal areas of Clayoquot Sound, BC with and without salmon farms.

Keywords: intertidal community, mussels, *Fucus*, phytoplankton biomass, salmon aquaculture

Abstract

Open net-pen salmon aquaculture releases organic and inorganic wastes to the local environment, providing a nutrient subsidy to many marine organisms. The availability of these wastes to adjacent intertidal communities is unknown. I examined productivity (as biomass/area or individual) in intertidal mussels, *Fucus* and phytoplankton using a three-level spatially nested design at five salmon farms and four reference locations throughout Clayoquot Sound during the summers of 2001/2. No significant effect of salmon farms on production of any of the organisms was detected, however non-significant but predicted trends were noted for *Fucus* biomass at two farms, and a reference that had been a farm previously for several years. Further investigation over 2-3 more years could resolve the question of whether this is a real trend. Most of the variation for all data was explained by small (meters) or large (kilometers) scale variation, whereas mesoscale (100's of meters) explained little. Possible reasons for no effect of farms on intertidal productivity are distance of farm from the intertidal zone, flushing rate by currents, and pulsed release of wastes from farms.

Introduction

Ecological productivity is the conversion of resources to consumer biomass, through growth of individuals or reproduction (Waide *et al.* 1999, Mittelbach *et al.* 2001). There is a consensus that factors regulating productivity are both "bottom-up" (the supply of resources), and "top-down" (the activity of consumers) forces (Power 1992, Hunter and

Price 1992, Nielsen 2001). Nutrient supply sets the limit for potential productivity (Rhyther and Dunstan 1971, Fretwell 1977, Hunter and Price 1992), while species interactions determine realized productivity (Carpenter *et al.* 1987, Abrams 1995, Polis and Strong 1996).

Studies investigating the effects of bottom up factors on productivity of intertidal organisms in their natural environment are limited. Logistical constraints restrict the manipulation of resource supply or primary productivity, leading researchers to take advantage of natural resource gradients (Bustamante *et al.* 1995*a*, Menge *et al.* 1997*a*, Nielson 2001) or subsidies (Duggins 1989, Powell *et al.* 1991, Wootton 1991, Bustamante *et al.* 1995*b*) across spatial scales ranging from meters to hundreds of kilometers. A clear east to west gradient of increasing nutrients, primary production, and biomass of grazers was found over thousands of kilometers around the coast of South Africa (Bustamante *et al.*1995*a*). Dominance patterns of algal species also changed with the gradient. Nutrients and nearshore phytoplankton biomass were studied as possible explanations for distinct differences in two intertidal communities (algae vs. invertebrate dominated) separated by 80km along the coast of Oregon (Menge *et al.* 1997*a*). Phytoplankton and organic particulates were always more concentrated at the filter feeder dominated community. Translocation experiments using mussels (*Mytilus edulis*) and barnacles (*Balanus glandula*) between Aleutian Islands with and without kelp found growth rates of these species to be increased 2-5x at islands with kelp (Duggins *et al.* 1989). Nutrients from seabird colonies affect the distribution, abundance and dominance of algal and lichen species in the high intertidal zone (Wootton 1991).

Salmon net-cage aquaculture may provide a logistically more convenient system in which to further the investigation of nutrient subsidies to intertidal organisms. Wastes released from salmon farms have been implicated in elevating production of intertidal organisms such as phytoplankton, suspension feeders and algae (Wallace 1980, Jones and Iwama 1991, Ahn *et al.* 1998, Troell *et al.* 1999). In British Columbia (BC), salmon net cage farms are concentrated in two areas, Clayoquot Sound and the Broughton Archipelago, thus providing replicable units within a spatially manageable area. Continuous waste output, and static position of salmon farms for multiple years could allow for the investigation of changes in species productivity, population dynamics and community structure.

Over a cultivation cycle of Atlantic salmon (*Salmo salar*), 67% of feed pellets added to open net pens is converted to salmon biomass (1.5 feed conversion ratio, Naylor *et al.* 1998), the balance is released directly to the ocean as solid and particulate waste, primarily in the form of salmon metabolic wastes and excess feed. During this study (2001-2003), BC had 83 operational farms and an average annual production of 67 700 tonnes (range 50 000-85 400 tonnes round wt) (BC Salmon Farmers Association), which averages 816 tonnes of fish per farm per year, and 270 tonnes of waste per farm per year. It is estimated that 70% of nitrogen (~100kg per 1000kg fish produced) and 80% of phosphorus (~20kg per 1000kg fish produced) added as feed are released to the environment in dissolved and particulate forms (Holby and Hall 1991, Hall *et al.* 1992, Valiela *et al.* 1992).

Integrating the culture of filter feeders (e.g. oysters or mussels) and macroalgae with salmon has been examined as a candidate solution to reducing the amount of dissolved and particulate wastes released to the surrounding environment with variable results (Wallace 1980, Jones and Iwama 1991, Stirling and Okumus 1995, Taylor *et al.* 1995, Ahn 1998, Troell and Nordberg 1998, Troell *et al.* 1999). Mussels (*Mytilus edulis*) growing on salmon farms were found to grow twice as fast as mussels growing subtidally and intertidally nearby (Wallace 1980). This result was attributed to a farm derived resource subsidy in the winter when phytoplankton densities are low. Chlorophyll *a* (Chl *a*), particulate organic matter (POM) concentrations, and growth rate of mussels were found to be higher near salmon farms when compared to mussel farms in Scotland (Stirling and Okumus 1995). They support Wallace (1980) and attribute elevated mussel growth rates to a year round subsidy of POM by salmon farms. Jones and Iwama (1991) varied the distance of hanging baskets filled with oysters from a salmon farm and found oyster growth to follow a Chl *a* and POM gradient which peaked at the farm. Two species of kelp have been shown to be efficient at removing dissolved nitrogenous wastes from salmon farms, showing a positive linear relationship between nutrient concentration and uptake (Ahn *et al.* 1998). In contrast, Taylor *et al.* (1995) found no effect of salmon farm wastes on mussel growth (condition index, crude protein and carbohydrate content), POM or Chl *a* concentration. However, they attribute these observations to dense populations of mussels growing on the farm utilizing any farm-derived POM or phytoplankton. Cheshuk *et al.* (2003) found no effect of culturing mussels on salmon farms compared to those cultured distant from the farm. They attribute these observations to a combination of ambient concentrations of phytoplankton and POM always exceeding the filtration capacity of mussels, mussels were cultured too far from the farm, and dilution of salmon farm derived POM by flushing.

The objective of this study was to determine if salmon farms provide a resource subsidy to intertidal organisms. Phytoplankton, the intertidal brown algae *Fucus distichus* and the intertidal mussel *Mytilus edulis* were chosen as indicator species for several reasons. All were present throughout Clayoquot Sound, are easily collected, and have been shown to increase productivity when grown near a salmon farm (Topinka and Robbins 1976, Ronnberg *et al.* 1992, Stirling and Okumus1995, Creed *et al.* 1997, Seguel *et al.* 2002). In addition, these organisms have important functional roles in the intertidal community. Phytoplankton is a critical food source for suspension feeders (Seed 1976), and *Fucus* and mussels are dominant occupants of limited primary space. *Fucus* provides resources to grazers as germlings (Lubchenco 1983), refuge for other algaes and invertebrates (both herbivores and predators) from desiccation (Menge 1978, Wootton 1991) as mature canopy-forming plants. Mussels are an important food source for intertidal predators such as seastars, whelks and marine birds. Additionally, altering the production of these organisms has been shown to have indirect effects at the community level, affecting species composition, abundance and interactions (Paine 1974, Menge 1978, Wootton 1991, Petraitis 1995). The prediction of this study was that phytoplankton, *Fucus* and mussels would show elevated productivity, measured as biomass, with proximity to salmon farms, the signal strength inversely proportional to distance from the farm.

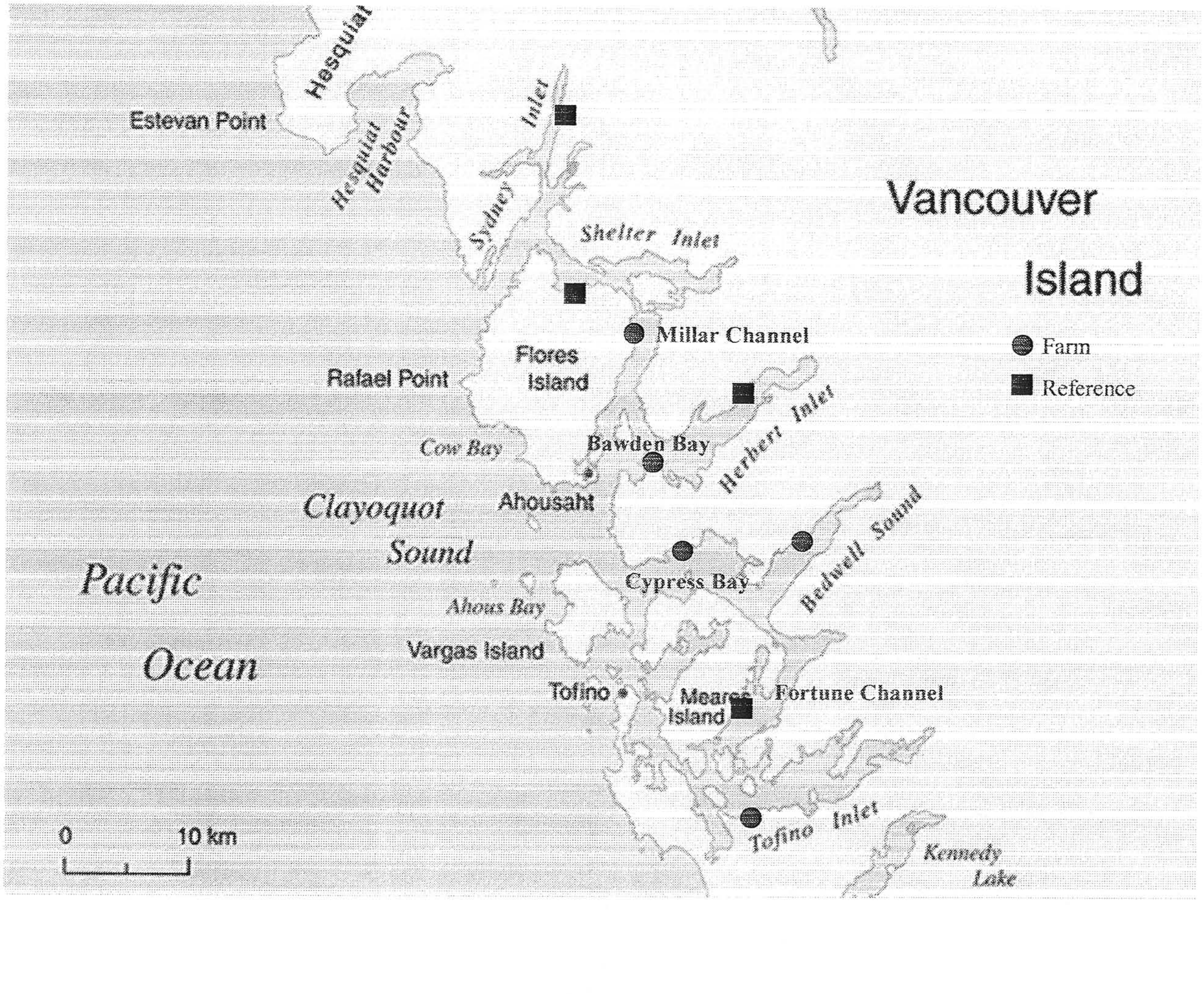
Methods

1) General Sampling Design

This study was conducted in the *Fucus* band of the mid-intertidal zone, adjacent to five salmon farms and four reference locations in Clayoquot Sound, BC during May-September of 2001 and 2002 (Figure 1).

I employed a three-level nested sampling design to determine the spatial scale at which ecological differences may be detected; locations (kms apart), sampling stations nested within locations (100s of m apart), and transects nested within sampling stations (10s of m apart). Locations were designated as treatments where there were farms, or references where there were no farms. Reference locations were meant to represent the natural ecological conditions (including variation) of Clayoquot Sound. Each location was assigned between three and six sampling stations, spaced approximately 200-2300 meters apart. Each farm had between four and six sampling stations. At farm locations, one sampling station was placed directly adjacent to the farm (50- 465m away), two to four others spaced approximately 200-800 meters apart up and downstream from the farm. The final sampling station was positioned 900-7000 meters away from the most distal sampling station to act as a within-location reference. Results from a pilot study indicated that reference stations should be located within the same inlet or channel as the farm in order to eliminate interlocation variation, which was usually the most significant source of variation. The same design was replicated at reference locations. The spacing of sampling stations is not perfectly replicated at all locations but the design is conserved. Sampling stations were chosen based upon accessibility to the researcher, similar slope and bearing with preference to small bedrock headlands. Three transects were established at each sampling station, spaced a few meters apart perpendicular to the waterline, their positions based on maintaining similar slope and bearing. Phytoplankton samples were collected in 2001. All other data were collected in 2002.

Figure 1. Location of study sights (Farm and Reference Locations) in Clayoquot Sound. Shelter Inlet was a farm location in 2001, but was harvested in the fall of 2001 and left fallow for 2002.



2) Sample Collection

a) Phytoplankton

Phytoplankton biomass (μg Chl *a*/L)was estimated from three replicate 2L samples collected in opaque HDPE (high density polyethylene) bottles at flooding or high tide directly over each transect, approximately 1m below the surface of the water. I collected these samples at high tide in order to reflect what was actually covering, and most likely available to the intertidal organisms. Subsamples of 500mL were filtered from each bottle under very low light onto 0.45μm pore size glass fiber filters, wrapped in foil (to prevent light exposure) and frozen at -20°C. This procedure was repeated in June, July and August 2001. Chlorophyll *a* (Chl *a*) was extracted from filtered samples in 12 mL of 95% ethanol for 18-24 hours at 4°C. Absorbance was measured at 649, 665 and 750 nm using an Ultrospec 2000 (Pharmacia Biotech) spectrophotometer under reduced light in a 10 cm cuvette, as per Wintermans and DeMots (1965). Chl *a* concentration (μg/L) was calculated from the following equation.

$$\text{Chl } a = [13.7(A_{665}-A_{750}) - 5.76(A_{649} - A_{750})][E/F(L)] \qquad \text{(eqn. 1)}$$

where E= extraction volume in mL, F= filtration volume in liters, L= cuvette length in cm, A_x= absorbance at wavelength x in nm (DeMots 1965).

b) *Fucus*

Fucus biomass was estimated using dry biomass from three replicate 10cm^2 quadrats placed one vertical meter below the top of the *Fucus* zone along each transect. Whole plants whose holdfasts were within the quadrat were collected. Samples were collected within one week (June 2002) to minimize growth between start and end of collection. Samples were rinsed in fresh water and any non-*Fucus* biomass (i.e. small invertebrates, attached algae) removed. Samples were frozen at -20 °C until such time as they could be dried at 55°C 1-4 days (until no further changes in mass occurred). Dried samples were weighed to the nearest 0.1g.

c) Mussels

Mussel production was estimated using dry biomass of mussels collected from three replicate 5 cm^2 quadrats contained within a 0.5 m^2 quadrat (one each in the top corners and one at the bottom center) placed one vertical meter below the top of the *Fucus* band at each transect. All samples were collected in July 2002. Individuals were counted and dried at 55 °C for 2-4 days (until no further changes in mass occurred). Individuals weighed in groups of 10 to the nearest 0.1g.

3) Data Analysis

I tested for an overall farm effect using independent samples t-tests with farms and reference locations as replicates.

In order to determine the spatial scale(s) most responsible for variation, I used a mixed model nested ANOVA (SPSS version 10.07, 2000) with treatments (farm/no farm) as fixed factors, locations, sampling stations and transects as random factors. I performed variance components analyses to quantify (percent) variation contributed from each spatial scale.

When a factor was significant, Student-Newman-Keuls post-hoc tests were performed to identify differences.

The ANOVA assumption for normality of residuals was checked using Q-Q plots. Homogeneity of variance of residuals was tested using Levene's test. Data was never transformed as there was never an instance when all data sets violated the assumptions of normality or equal variance.

Power Analysis

To investigate the possibility of type ll error (failing to reject the null hypothesis when it is false) I performed a post-hoc power analysis for t-tests of treatment effects. I determined the minimum effect size detectable given my sample size, and calculated the minimum sample size required for the observed effect size to be significant (Zar 1999).

Results

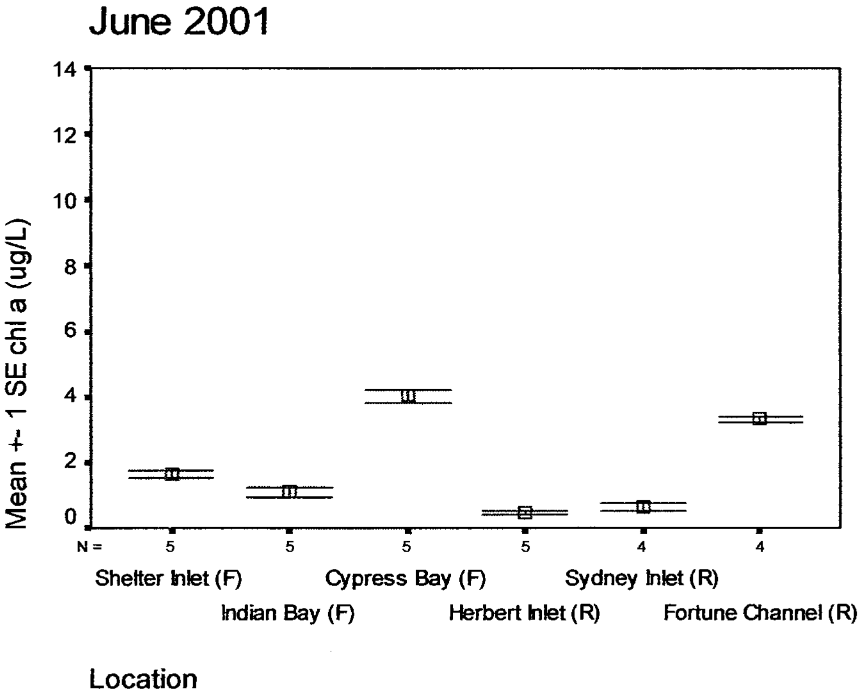
a) Phytoplankton

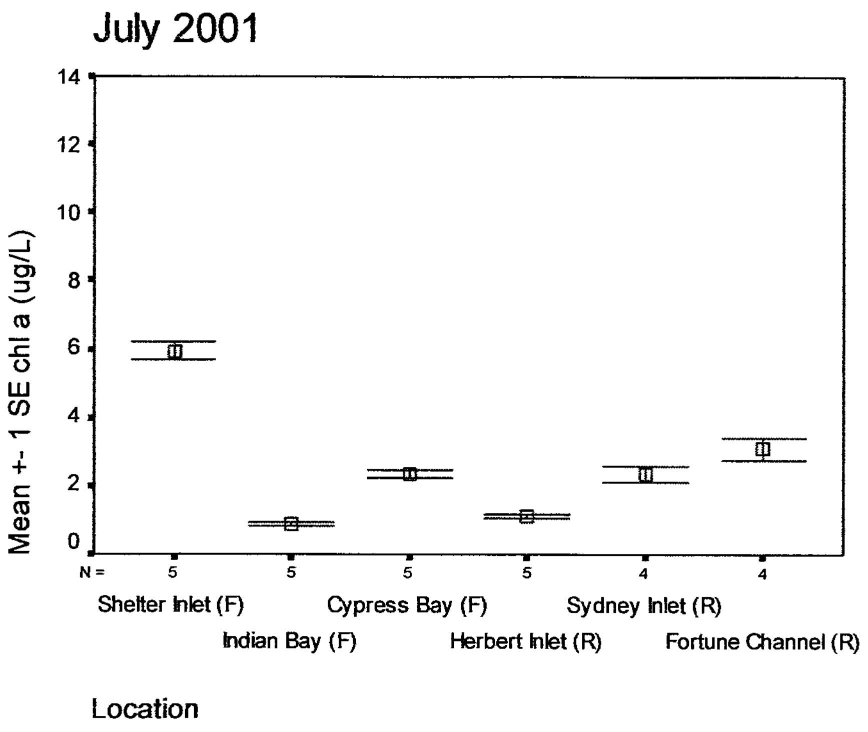
Phytoplankton biomass (μg Chl *a*/L) in the intertidal zone does not appear to be influenced by the presence of a salmon farm on any spatial scale considered in this study. Mean chlorophyll *a* (Chl *a)* concentration at farm locations was not significantly different from reference locations (Table 1). There was significant variation among locations for each sampling event (Table 1), but no consistency to location mean rank temporally or spatially and no trends emerged to indicate elevated Chl *a* concentrations at farms (Figure 2). In June, location averages ranged 0.5-4 ug/L Chl *a*, Cypress Bay and Fortune Channel 2-4x higher than the other locations (Figure 2). In July, location averages ranged 0.5-6.0 μg/L Chl *a*, Shelter Inlet 3-6x higher than the other locations (Figure 2). In August, location averages ranged 0.5-11.0 μg/L Chl *a*, Sydney Inlet 2-20x higher than the other locations (Figure 2). Within farm locations, phytoplankton biomass was not influenced by distance from the farm (Table 1). ANOVA for each location during each sampling event were largely not significant, and no trends emerged to indicate elevated phytoplankton biomass when close to farms (Figure 2). The only significant intralocation variation for phytoplankton biomass were reference locations, the one exception being the farm in Tofino Inlet in June, but the predicted gradient was not observed. For all sampling events, location was the largest source of variation (Table 1). Transect accounted for most of the remaining variation except in August when treatment (farm/no farm) accounted for 15%. Chl *a* concentrations never exceeded 7.0 ug/L for any sample (n=84), except in Sydney Inlet during August (n=4), when the average was about 11 ug/L and peaked at 14 μg/L. Tofino Inlet was consistently below 2.0 μg/L for all samples. Overall phytoplankton biomass increased over the three sampling periods but this trend was not significant ($F_{(2,16)}$=1.237, p=0.317).

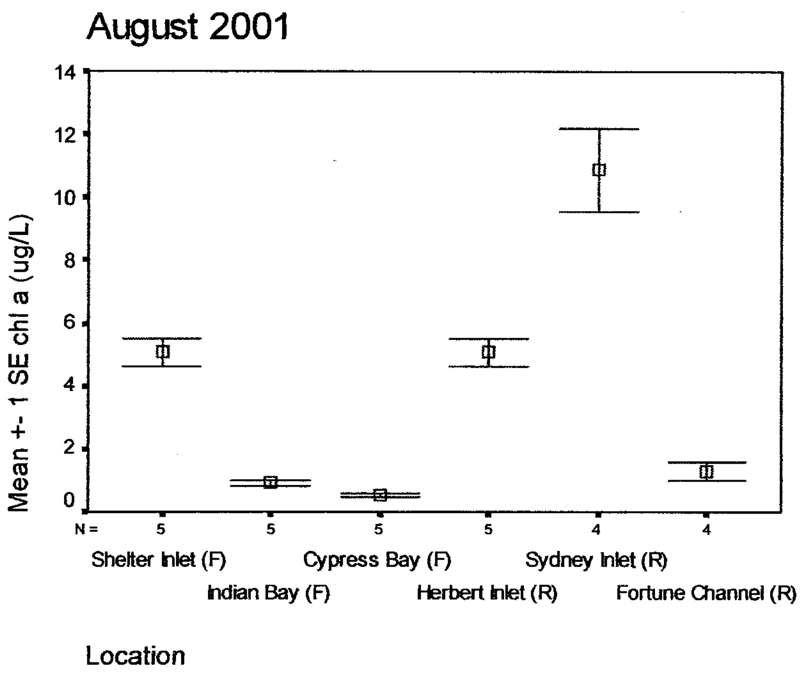
Table 1. Evaluation of the effects of treatment (farm/no farm), location, and distance from farm (sampling station) on Chl *a* (μg/L), *Fucus* dry weight (g/10cm^2) and mussel dry weight (g/mussel) using nested ANOVA and variance components analysis (sampling station nested within location, location nested within treatment). Replicate samples were taken from three transects at each sampling station. Each effect above transect (sampling station, location, treatment) was tested by using the MS of the next lowest factor as the error term.

Variable	Source of variation	df	MS	F	p	Variance component
June Chl *a*	Treatment	1	12.805	0.381	0.573	0%
	Treatment(location)	4	33.757	139.35	0.000	93%
	location(sampling station)	22	0.243	1.68	0.061	1.4%
	Error	55	0.144			5.4%
July Chl *a*	Treatment	1	16.25	0.285	0.622	0%
	Treatment(location)	4	57.45	110.57	0.000	85%
	Location(sampling station)	22	0.520	0.724	0.796	0%
	Error	56	0.718			15%
August Chl *a*	Treatment	1	264.08	1.414	0.300	9%
	Treatment(location)	4	187.91	47.811	0.000	77%
	Location(sampling station)	22	3.93	2.37	0.005	5%
	error	56	1.67			9%
Fucus dry wt.	Treatment	1	20.277	0.04	0.848	0%
	Treatment(location)	7	518.91	16.36	0.000	48%
	Location(sampling station)	28	31.72	0.721	0.832	0%
	error	74	44.00			52%
Mussel dry wt.	Treatment	1	0.000	0.005	0.947	0%
	Treatment(location)	7	0.121	5.91	0.000	38%
	Location(sampling station)	27	0.022	1.80	0.034	15%
	error	52	0.012			46%

Fig 2. Average (+/- SE) Chl *a* (μg/L) for sampling stations within each location in June, July August 2001. Each sampling station is represented by three replicate samples. There is no consistency to Chl *a* concentration temporally or spatially, except for Tofino Inlet, which was consistently below 2 μg/L. F= farm, R= reference.


June 2001


July 2001
Mean +- 1 SE chl a (ug/L)
14
12
10
8
6
4
2
0
N =
5
5
5
5
4
4
Shelter Inlet (F)
Indian Bay (F)
Cypress Bay (F)
Herbert Inlet (R)
Sydney Inlet (R)
Fortune Channel (R)
Location


August 2001
Mean +- 1 SE chl a (ug/L)
14
12
10
8
6
4
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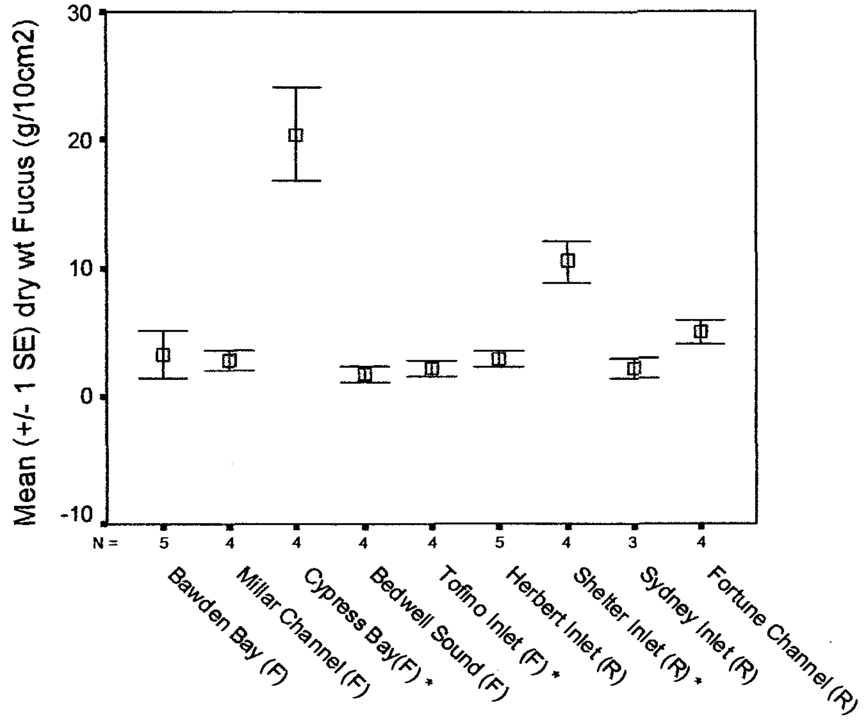
0
N =
5
5
5
5
4
4
Shelter Inlet (F)
Indian Bay (F)
Cypress Bay (F)
Herbert Inlet (R)
Sydney Inlet (R)
Fortune Channel (R)
Location

b) *Fucus*

Average dry biomass of *Fucus* (g/10 cm^2) collected at farms was not significantly different from that at reference locations (Table 1). There was significant variation among locations (Table 1) but no trends emerged indicating elevated *Fucus* biomass at farm locations relative to reference locations (Figure 3). Cypress Bay (farm) and Shelter Inlet (reference) had the highest mean values and had 2-20x more *Fucus* that the other locations (Fig. 3). Distance from farm had no significant effect on *Fucus* biomass (Table 1), however, predicted non-significant trends were observed at two farms (Fig 3). Cypress Bay and Tofino Inlet showed increasing *Fucus* biomass as distance from the farm decreased. These locations are anchored 170m and 50m directly perpendicular to the shore respectively. Notably, Shelter Inlet (reference) had the second highest mean *Fucus* biomass, and showed the predicted (although nonsignificant) gradient, possibly because for several years until the fall of 2001, a farm was located there. These results may be a reflection of previous long-term exposure. The farm at Shelter Inlet was 190m from shore. Variation among transects and location were the primary sources, 52% and 47% respectively (Table 1).

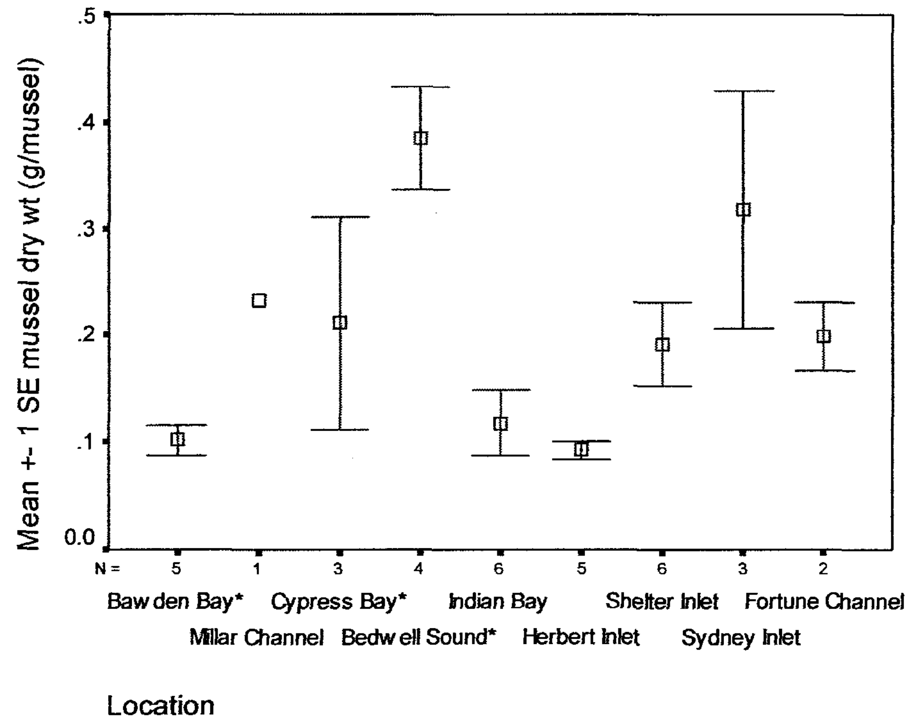
Figure 3. Average (+/- 1 SE) dry weight of *Fucus* (g/10cm^2) for all locations. F = farms, R = references. Each location is represented by three to five sampling stations. Marked locations (*) are where the diminishing gradient was observed, and indicated as a negative correlation coefficient (r)). Correlation coefficients for distance of sampling stations to farm and *Fucus* biomass for each farm location are: Bawden Bay r= 0.503, r^2= 0.253, Millar Channel r= 0.608, r^2= 0.369, Cypress Bay r= -0.68, r^2= 0.462, Bedwell Sound r= 0.464, r^2= 0.215, Tofino Inlet r= -0.56, r^2= Shelter Inlet r= -0.861.



c) Mussels

Average dry biomass of mussels (g/m^2) collected at farms was not significantly different from that at reference locations (Table 1). There was significant variation among locations (Table 1), but no trend emerged showing mussels to have elevated biomass when growing near a farm (Figure 4). Distance from farm had no significant effect on mussel biomass (Table 1). However, at three farms (Cypress Bay, Bawden Bay and Bedwell Sound, which were 170, 285 and 400m from shore respectively), mussels collected closest to these farms had higher average dry biomass than those growing further away. The predicted diminishing gradient was not observed. Variation among transects and location accounted for most of the variation, 46% and 38% respectively.

Figure 4. Mean (+/- SE) dry weights of *Mytilus edulis* (g/m^2) for each location. (F) = farms, (R) = references. Each location is represented by 1-6 samples. Marked locations (*) are where mussels collected closest to farms had the highest average dry wt. The predicted gradient was not observed at any farm location.



d) Power Analysis

Table 2 summarizes the results of the post-hoc power analysis for treatment effects. Effect sizes would need to be 6-24x larger than observed in order to be significant. In most cases, sample sizes required for the observed effect sizes to be significant far exceed the number of operational salmon farms in Clayoquot Sound, which is approximately 20.

Table 2. Post-hoc power analysis for the effect of treatment (farm/no farm) on productivity of intertidal phytoplankton (μg/L), *Fucus* (g/10cm^2) and mussels (g/individual) throughout Clayoquot Sound. Minimum significant effect size and minimum sample size required for observed effect size to be significant are reported.

Variable	N	Observed effect size	Min. sig. effect size	Min N for sig. obs. effect Size
[Chl *a*] June 2001	3 farms 3 refs	0.78 μg/L	4.79 μg/L	65
[Chl *a*] July 2001	3 farms 3 refs	0.87 μg/L	5.98 μg/L	82
[Chl *a*] Aug 2001	3 farms 4 refs	3.31 μg/L	9.85 μg/L	19
Fucus dry wt.	5 farms 4 refs	0.92 g	14.77 g	808
Mussel dry wt.	5 farms 4 refs	0.01g	0.24 g	1727

Discussion

Phytoplankton

Phytoplankton biomass varied across locations and sampling periods but was not significantly different at farms compared to reference locations. This is due to the large effect of location (June 94%, July 85%, Aug 69%). This implies that spatial and temporal differences among locations are more influential upon phytoplankton biomass than are nutrients from salmon farms, and that each location should be examined independently. Physical and oceanographic characteristics of each location (i.e. depth and width of channel/inlet, bathymetry, shoreline morphology, directional orientation) interact with currents and wind to affect nutrient availability, residence time of water, temperature and salinity (Mackas *et al.* 1980, Thompson 1981, Mackas *et al.* 1985, Duarte 1990, Eslinger *et al.* 2001), all important determinants of phytoplankton production and distribution (Mackas *et al.* 1985, Daly and Smith 1993, Kocum *et al.* 2002).

No effect of distance from farm was found. This result is supported by other studies that found no increase in phytoplankton biomass near salmon farms (Taylor *et al.* 1992, Stirling and Okumus 1995, Mazzola and Sara 2001, Cheshuk *et al.* 2003). This may be due to a number of factors, both physical and biological. First is the question of availability of salmon farm derived nutrients to phytoplankton. Currents may be flushing dissolved wastes from the farm sites and surrounding area before local phytoplankton can take advantage of the subsidy. Additionally, the direction of current flow may not be toward the intertidal zone, thus preventing both farm derived nutrients and any additional phytoplankton around farms from being transported to the intertidal zone. Currents can also alter the density of nutrients and algal patches by dispersion (Mackas *et al.* 1985), thus altering the magnitude of algal production and its distribution. Menge *et al.* (1997*a*) provide evidence that nutrients can vary haphazardly at small spatial scales (100's of meters) and over short time periods (days), likely due to natural variation in small scale current patterns and temperature (Daly and Smith 1993).

A third explanation is the effect of consumers on phytoplankton abundance. Consumption by mussels growing on the farm or in the intertidal zone can prevent phytoplankton biomass from accumulating. Other researchers have suggested that mussels growing on salmon farms prevent phytoplankton from surpassing ambient levels (Taylor *et al.* 1992) by virtue of their ability (in high densities) to significantly deplete particulate matter from the water column (Cloern 1982, Wildish and Kristmanson 1984, Frechette and Bourget 1985a,b, Asmus and Asmus 1991). If this was the case in the intertidal zone, then mussel biomass could reflect this. This possibility will be discussed below. Additionally, herbivorous micro and macrozooplankton and are key consumers of phytoplankton and are capable of

removing large proportions of phytoplankton biomass in the coastal North Pacific (Mackas *et al.* 1980, Daly and Smith 1993, Yin *et al.* 1996, Strom *et al.* 2001).

Fucus

No significant differences were found for *Fucus* biomass between farms and reference locations. Unlike the patterns observed for phytoplankton biomass, *Fucus* biomass was fairly consistent among locations. Although location was a significant source of the overall variation (47%), it is explained largely by Cypress Bay and Shelter Inlet being significantly more productive than the other locations. This pattern could be caused by nutrient subsidy from salmon farms, because both of these locations show *Fucus* to be more abundant near the farm at Cypress Bay, or the fallow farm at Shelter Inlet, than farther away.

Predicted but nonsignificant trends for *Fucus* biomass were detected at two of five farms (Cypress Bay and Indian Bay) and one reference location (Shelter Inlet) that was a recently (7-8 months) fallowed farm. *Fucus* is a perennial algae that can live 5-6 years, thus the pattern of biomass distribution at the fallow farm could be a reflection of previous long term exposure. These observations are in agreement with other studies that found another species of *Fucus* (*F. vesiculosis*) to show more rapid growth near salmon farms (within 50m) relative to plants grown further away (200m and 700m) (Ronnberg 1992).

The predicted pattern was not observed at all farm locations. Again, this raises the question of availability of salmon farm derived nutrients to intertidal *Fucus* at these locations. It is possible that currents are not delivering nutrients from salmon farms to the intertidal zone (Cheshuk *et al.* 2003).

Another possibility is competition from other algal species. Although additional nutrients (mainly nitrogen) elevate *Fucus* growth (Thom 1983, Ronnberg 1992, Worm and Sommer 2000), the combination of elevated nutrients and the presence of competitors can result in depressed *Fucus* growth (Thom 1983, Worm and Sommer 2000). Other co-occurring species of algae, mainly microalgae and filamentous macroalgae, are adapted for rapid nutrient uptake and growth, whereas perennial canopy-forming algae like *Fucus*, have slower nutrient uptake and growth rates (Wallentinus 1984, Worm and Sommer 2000). Nutrients are depleted by other competitively superior species, or epiphytes of *Fucus* reduce light availability and compete for nutrients and CO_2/O_2. *Fucus* has been cited as growing best where there is little competition from other organisms (Edelstein and McLachlan 1975, Thom 1983). Studies documenting the decline of *Fucus* due to sewage pollution note concurrent elevations in phytoplankton biomass, epiphyte and periphyton growth (Kangas 1982, Thom 1983). Green filamentous algae (mainly *Acrosiphonia coalita*: Cladophoraceae) were visibly abundant only in Bawden Bay and Shelter Inlet but it is not clear if they are displacing *Fucus*. *Fucus* was abundant throughout Shelter Inlet relative to other locations, but the farm was fallow and thus not releasing dissolved wastes. The presence of abundant annual green algae that have not been exposed to salmon farm wastes suggests that other factors are determining the level of productivity in Shelter Inlet. The abundance of *Fucus* at Bawden Bay was not different from other locations where filamentous green algaes were not present or visibly abundant. This could be explained by annual variation in nutrient availability which will affect the abundance and distribution of annually growing algae more than perennials. *Fucus* is resilient to seasonal and short term variation in growth factors (Edelstein and McLachlan 1975), possibly due to its ability to store nitrogen and continue growing for weeks under stressed conditions (Pedersen and Borum 1996). Other species of intertidal algae co-occurred with *Fucus*, and most samples of *Fucus* had epiphytes, but neither was quantified for this study.

Aside from Cypress Bay, *Fucus* biomass did not vary appreciably among the remaining locations. In areas of Millar Channel and Bedwell Sound where *Fucus* was visibly sparse, no other algal species were present. This observation could indicate that dissolved nutrients from salmon farms are not subsidizing adjacent intertidal zones in Bedwell Sound and Millar Channel. *Fucus* is adapted to live in low nutrient levels where fast growing filamentous green algaes cannot (Pedersen and Borum 1996).

Significant spatial variation occurred at the smallest (meters) scale, reflecting natural small scale patchiness in *Fucus* growth and abundance, maintained by physical scouring, temperature and salinity (Edelstein and McLachlan 1975, Munda 1977), wave exposure and nutrients (Thom 1983), competition for primary space and past recruitment history (Creed *et al.*1997), and intertidal height (Keser and Larson 1984). Although small scale variation describes half of the overall variation, I believe it is an accurate representation of the natural distribution of *Fucus* biomass, as it was consistent within and among seven of the nine locations.

The pattern of *Fucus* biomass distribution contrasts that of phytoplankton. Elevated *Fucus* biomass may be a reflection of constant exposure to salmon farm wastes accumulated over several seasons or years whereas phytoplankton is short lived (days to weeks) and only reflects short term changes in nutrients. Additionally, phytoplankton is pelagic, its distribution and density are subject to dispersal by currents over short time scales (Mackas *et al.* 1985, Denman and Garrett 1983, Mackas *et al.* 1980). In contrast, *Fucus* is an attached benthic algae whose distribution and density are fixed over the time scale encompassed by this study. Additionally, consumers augment phytoplankton abundance, whereas *Fucus* is unpalatable as adult plants and is generally not consumed (Lubchenco 1983).

Mussels

Average dry weight of mussels growing near salmon farms was not significantly different from those growing at reference locations. Location explains much of the overall variation (38%) indicating that mussel growth parameters are variable among locations and each location should be investigated independently. The main factors affecting mussel growth are food availability, salinity, and temperature (Seed 1969, Freeman and Dickie 1979, Widdows *et al.* 1979, Stirling and Okumus 1985, Page and Hubbard 1987) which can vary over small spatial scales (Stirling and Okumus 1995, Menge *et al.* 1997). Phytoplankton biomass was shown to be variable among locations and throughout the summer of 2001, thus indicating that food availability can be variable, and possibly near growth limiting levels (Widdows *et al.* 1979, Stirling and Okumus 1995). The influence of fresh water was observed at numerous places throughout Clayoquot Sound. Mussels growing in the mouth of streams, or close by (within a few meters) were visibly larger than those growing a few meters further away. The most striking example of this effect was at one sampling station in Millar Channel, where a creek drained approximately 10 m away on either side. The mussels in the creek mouths were visibly much larger than those growing at the intervening sampling station. However, salinity was not measured in this study thus differences among locations are unknown. Temperature was also not measured during this study.

Distance from farm also had no significant effect on mussel growth. However at three farms (Cypress Bay, Bawden Bay and Bedwell Sound), mussels taken from the sampling station closest to each farm were larger than those further away. These results are similar to earlier studies (Wallace 1980, Jones and Iwama 1991, Stirling and Okumus 1995) that found elevated growth of mussels and oysters up to 500m away from salmon farms. However these studies also documented elevated POM and Chl *a* concentrations in association with elevated bivalve growth. Although in 2001 I did not observe any increase in phytoplankton biomass associated with farms, consumption by intertidal mussels could be masking this effect. Ingestion of farm derived resources (POM or phytoplankton stimulated by salmon farm wastes) could be confirmed by stable isotope analysis, which will be addressed in another part of this study (Chapter 3).

Two farms did not show predicted gradients. These results are similar to other studies investigating the potential for integration of bivalve and salmon culture that found no measurable increase in bivalve growth (Taylor *et al.* 1992, Cheshuk *et al.* 2003). Taylor *et al.* (1992) attribute the lack of an effect to dense populations of mussels growing on the net pens removing any farm-derived POM or phytoplankton. Cheshuk *et al.* (2003) found no effect of culturing mussels near salmon farms (70 and 100m) compared to those cultured distant from the farm (500 and 1200m). They attribute these observations to a combination of ambient concentrations of phytoplankton and POM always exceeding the filtration capacity of mussels, mussels were cultured too far from the farm, and dilution of salmon farm derived POM by flushing. These same explanations likely apply to this study. Mussels growing on the farms may have already intercepted any farm generated POM or phytoplankton, the intertidal zone could be too far from farms for mussels to intercept any farm generated POM or phytoplankton, or currents may be transporting POM and phytoplankton away from the intertidal zone. Additionally, other research has demonstrated that transport of solid particulates are largely vertical and within 60m (variable depending upon local current speeds and directions and depth) of the edges of the cages (Brown *et al.* 1987, Kaspar *et al.* 1988, Sutherland *et al.* 2001, Cheshuk *et al.* 2003). The farms used in this study ranged 50-400m away from the intertidal zone, thus meeting or exceeding the spatial limit for solid waste dispersal described by other researchers. Uneaten pellets are known to fall to the seafloor largely intact, thus not contributing to suspended particulate matter (Cheshuk *et al.* 2003).

It is possible that mussels are not food limited in Clayoquot Sound, leaving other growth factors to explain the differences among locations for mussel size. Maximum filtration rates for mussels 1.7 cm long is 2.6 ug/L seston (algal plus detrital particulate matter) (Widdows *et al.* 1979). The average length of mussels in the present study was less than 1.7 cm long (data not shown). About half the samples in this study met or exceeded 2.6 ug/L of Chl *a* in 2001, and no location was consistently below 1.0 ug/L. Considerable growth by mussels can be maintained at 1.0 ug/L seston at a commercial mussel culture operation (Stirling and Okumus 1995). Although seston concentration was not measured in this study, Chl *a* concentrations alone appeared to be at feeding saturation levels for at least half the samples in 2001. It is unknown whether non-algal seston elevates total food availability to filtration limits in the remaining samples. Although Chl *a* measurements are from the year before mussel collection, the information provides evidence that food availability is variable over a short time period, ranging from potentially growth limiting levels, to exceeding maximum filtering capacity, thus possibly contributing to variation in mussel size. If intertidal mussels are not food limited in Clayoquot Sound, additional resources from salmon farms are unlikely to have an effect on biomass. However, stable isotope analysis could give a clearer picture of food sources and is the focus of the next chapter.

Small scale (among transects) variation explains much (46%) of the overall variation in mussel biomass and is in agreement with other studies (Seed 1969, Menge 1978, 1991, Blanchard and Bourget 1999). This is likely a reflection of natural small scale patchiness of mussels caused by numerous interacting physical and biological processes such as topographical heterogeneity (Blanchard and Bourget 1999, McKindsey and Bourget 2000), scouring by logs (Dayton 1971), predation (Menge 1976, 1978), recruitment history (Menge 1991), growth and competition (Menge 1991, Hunt and Scheibling 2001).

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Chapter 3.

Comparison of stable isotope (^{15}N) composition of intertidal eelgrass, *Fucus*, and mussels collected from areas of Clayoquot Sound, British Columbia, with and without salmon farms.

Keywords: stable isotope, ^{15}N, salmon aquaculture wastes, intertidal community, resource subsidy

Abstract

In British Columbia (BC), open net-pen salmon farms release an average of 270 tonnes/year/farm of organic and inorganic wastes, which are sources of nutrients to marine plants and animals. The extent to which this nutrient subsidy is available to intertidal organisms is unknown. The stable isotope ^{15}N was used as a tracer of salmon farm derived nutrients into adjacent intertidal communities at five farms and four reference locations in Clayoquot Sound, BC. Salmon feed and muscle tissue were significantly more enriched for ^{15}N than eelgrass, *Fucus* and mussels. However eelgrass, *Fucus* and mussels were not significantly different from one another, occupying the same range of 6-10 ‰ ^{15}N. Samples of eelgrass and *Fucus* often were more enriched than mussels. Although not significant, eelgrass, *Fucus* and mussels growing near salmon farms were on average enriched for ^{15}N in comparison to reference locations. No effect of distance of farm was found for eelgrass or *Fucus*. Mussels collected directly from farm net cages showed no enrichment in comparison to mussels collected from pristine areas. Explanations for no detectable effects are large amounts of natural variation, minimal contribution of wastes to diets of intertidal organisms due to distance of farms from intertidal zones, or dilution of wastes by currents and pulsed feeding of salmon.

Introduction

In British Columbia (BC), salmon net-cage aquaculture practices external waste management, allowing excess feed and salmon metabolic wastes to flow freely from the net cages into the surrounding environment. These wastes are potential food sources for both marine plants and animals. Dissolved nitrogenous wastes (mainly ammonia and nitrates) are used by phytoplankton and macroalgae (Jones and Iwama 1991, Ahn *et al.* 1998, Vizzini and Mazzola 2004), particulate matter by filter feeders and fish (Wallace 1980, Jones and Iwama 1991, Mazzola and Sará 2001). Organic enrichment from settled farm wastes results in significant changes in biological, physical and chemical features of the benthos below farms (Brown *et al.* 1987, Ye *et al.* 1991, Sutherland *et al.* 2001). The extent to which these wastes are assimilated into other local food webs is less well known.

There is compelling yet inconclusive evidence documenting increased growth or abundance by marine organisms in response to salmon farm wastes (Wallace 1980, Jones and Iwama 1991, Stirling and Okumus 1995, Ahn *et al.* 1998). The growth rate of mussels growing on a salmon farm in Norway was twice that of subtidal and intertidal populations distant from the farm (Wallace 1980). Mussels on the farm grew continually throughout the year, allowing individuals to reach double the size of the other populations. This result was attributed to a continuous food subsidy from the salmon farm, which allowed mussels to grow throughout the winter when growth would normally stop. However, neither algal biomass nor suspended particulate organic matter (POM) were measured.

Growth rates of Pacific oysters (*Crassostrea gigas*), concentrations of chlorophyll *a* (Chl*a*) and POM were compared between a Chinook salmon (*Oncorhynchus tschawytscha*) farm and two oyster farms (Jones and Iwama 1991). Chl*a* and POM concentrations increased with proximity to the salmon farm as did oyster growth. Results were attributed to a continuous and elevated supply of Chl*a*, POM and dissolved nutrients, generated by the salmon farm. However, differences in oyster densities between the salmon and oyster farm sites were not considered. The lower Chl*a* and POM concentrations observed at the oyster farm could be due to depletion by oyster feeding, rather than because of a decreasing gradient of particulate matter originating from the salmon farm.

Mussels were found to grow larger and more continuously throughout the year when grown near salmon farms than at commercial mussel farms in Scotland (Stirling and Okumus 1995). Higher concentrations of algal biomass and POM at salmon farms throughout the year likely accounted for this difference. However, differences in mussel density between the salmon and mussel farms were not considered, which could account for differences in particulate matter as mussels are efficient at depleting particulate matter from the water column (Cloern 1982, Wildish and Kristmanson 1984, Frechette and Bourget 1985). Since many more factors in addition to nutrient availability can affect productivity of marine plants and animals, a method tracing the assimilation of salmon farm derived nutrients by organisms would be beneficial.

Stable isotope analysis offers a natural method for tracing the flow of nutrients from a source through a food web. The heavy stable isotope of nitrogen (^{15}N) indicates the trophic position of organisms within their food web. ^{15}N is preferentially retained (fractionation) during metabolic reactions over the lighter and more common isotope ^{14}N and bioaccumulates on average 3-5$^{0}/_{00}$ per trophic level (DeNiro and Epstein 1981, Minagawa and Wada 1984, Peterson and Fry 1987). Stable carbon isotope ^{13}C composition ($^{0}/_{00}$) can be used to identify relationships between consumers and their resources as it does not fractionate to the same degree ^{15}N does. Slight fractionation occurs (1.0 -1.5 $^{0}/_{00}$), thus the $^{13}C/^{12}C$ ratios of consumers reflect that of their resources plus a slight enrichment (DeNiro and Epstein 1981, Peterson and Fry 1987). The ratio of heavy to light isotopes can be measured precisely with a mass spectrometer (Peterson and Fry 1987).

Stable isotopes have been used as natural tracers of the flow of nutrients between adjacent ecosystems (Kline *et al.* 1990, 1993, Reimchen *et al.* 2003), to indicate trophic position of organisms (Welch and Parsons 1993, Cabana and Rasmussen 1996, Vander Zanden and Rasmussen 1999, Vander Zanden *et al.* 1999), and trace the movements of animals (Hansson *et al.* 1997). Stable isotopes of carbon and nitrogen have been used to trace the fate of wastes released from salmon farms (Ye *et al.* 1991, Mazzola and Sara 2001, Vizzini and Mazzola 2004), providing vital evidence that salmon farm wastes are utilized by marine organisms.

The extent to which salmon farm wastes are assimilated into adjacent intertidal food webs is largely unknown. The focus of this study was to utilize the stable isotope ^{15}N to indicate usage of salmon farm derived nutrients by intertidal organisms. The focal organisms were two species of primary producers, a brown algae *Fucus distichus*, and eelgrass (*Zostera marina*), and a primary consumer, the blue mussel (*Mytilus edulis*). These species were chosen because they were easily accessible, can use salmon farm wastes directly, are long-lived and *Fucus* and mussels were present in all study areas. Short-lived organisms such as phytoplankton and zooplankton are sensitive to short term fluctuations in nitrogen availability which can result in significant variation in $\delta^{15}N$ at the base of the food web (Klein *et al.*1993, Cabana and Rasmussen 1996). Larger-bodied, long-lived organisms have slower tissue turnover rates and are less sensitive to short term seasonal variation in ^{15}N, thus providing a better picture of the baseline $\delta^{15}N$ of the food web.

Cultured salmon, their feed and wastes are isotopically enriched for ^{15}N relative to primary producers and consumers (Minagawa and Wada 1984, Welch and Parsons 1993) and their usual food sources. Over a cultivation cycle, 67% of pelleted feed added to net cages is converted to salmon biomass (1.5 feed conversion ratio, Naylor *et al.* 1998), the remainder is released to the local environment as dissolved and particulate wastes, primarily as salmon metabolic waste and excess feed. For the duration of this study (2002) there were approximately 83 farms in operating in BC, with an annual production of 67 700 tonnes of salmon (BC Salmon Farmers Association). On average each farm produces 270 tonnes of waste per year. Therefore, I predicted that intertidal primary producers and consumers utilizing salmon farm derived nitrogen should reflect this subsidy with enriched ^{15}N signatures relative to those without access.

Methods

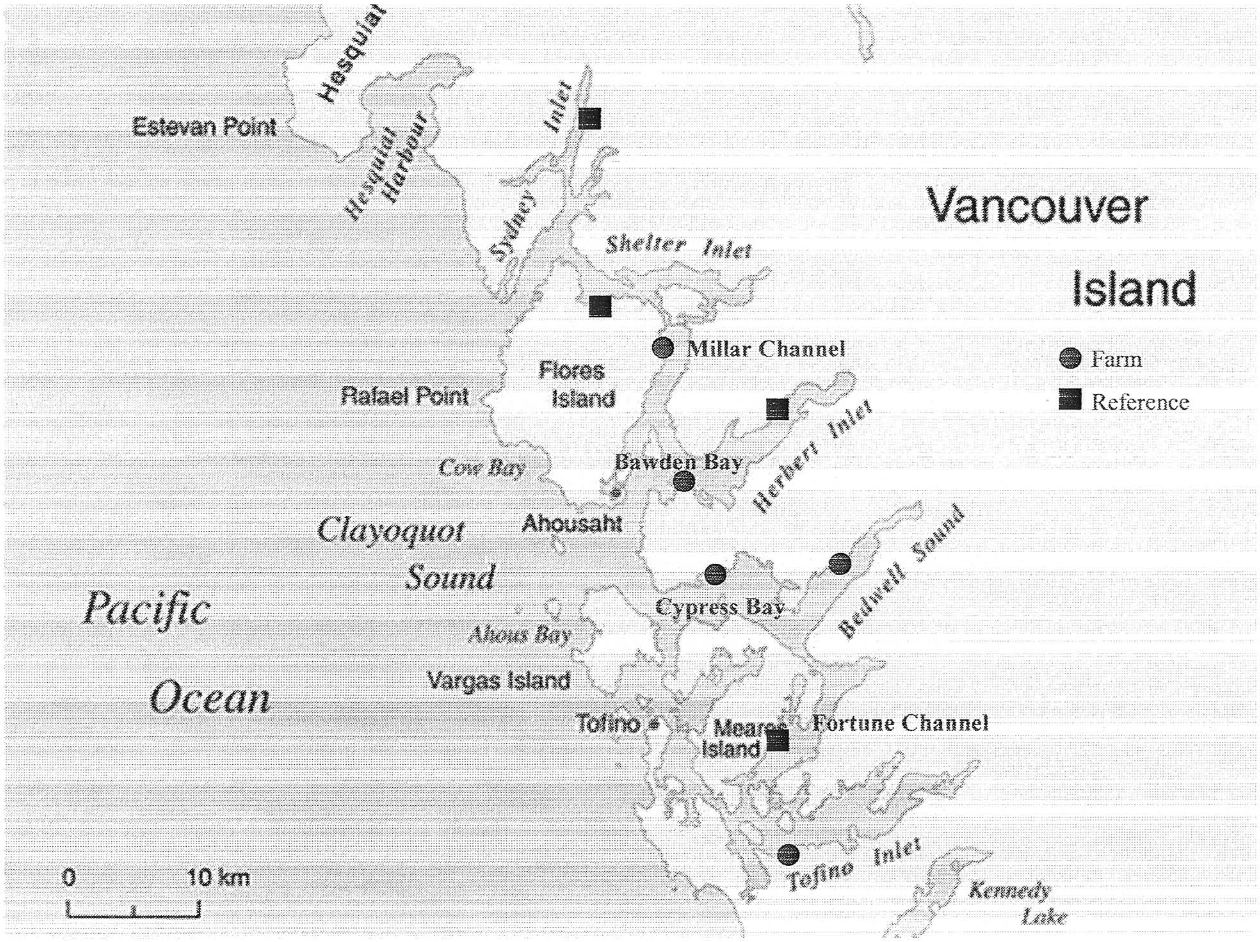
1) General Sampling Design

All aspects of this study were conducted in the *Fucus* band of the mid-intertidal zone, adjacent to five salmon farms and four reference locations in Clayoquot Sound, British Columbia during May-September 2001-2003 (Figure 1).

I employed a three-level nested sampling design to determine the spatial scale at which ecological differences may be detected; locations (kms apart), sampling stations nested within locations (100s of m apart), and transects nested within sampling stations (10s of m apart). Locations were designated as treatments where there were farms, or references where there were no farms. Reference locations were meant to represent the natural ecological conditions of Clayoquot Sound. Each location was assigned between three and six sampling stations, spaced approximately 200-2300 meters apart. All farms had between four and six sampling stations. At farm locations, one sampling station was placed directly adjacent to the farm (50- 465m away), two to four others spaced approximately 200-800 meters apart up and downstream from the farm. The final sampling station was positioned 900-7000 meters away from the most distal sampling station to act as a within-location reference. Results from a pilot study indicated that reference stations should be located within the same inlet or channel as the farm in order to account for interlocation variation, which was usually the most significant source of variation. The same design was replicated at reference locations. The spacing of sampling stations is not perfectly replicated at all locations but the design is conserved. Sampling stations were chosen based upon accessibility to the researcher, similar slope and bearing; most often on small bedrock headlands.

Three transects were established at each sampling station. Transects were spaced a few meters apart perpendicular to the waterline, their positions based on maintaining similar slope and bearing.

Figure 1. Location of study sights (Farm and Reference Locations) in Clayoquot Sound. Shelter Inlet was a farm location in 2001, but was harvested in the fall of 2001 and left fallow for 2002.



2) Sample Collection and Preparation

Salmon Farm Wastes

Two types of pelleted adult salmon feed (EWOS and Moore-Clarke), and muscle tissue from three adult BC farmed Atlantic salmon were used as indicators of the ^{15}N signature of the wastes from salmon farms. Salmon muscle tissue was used instead of actual metabolic wastes because whole fish could not obtained, and the ^{15}N signature of the wastes are generally only slightly lower than that of the tissues (Owens 1987). Feed samples were obtained from unopened bags; salmon were obtained from three different retail fish markets. All samples were dried at 65°C (2-4 days) until no further change in mass occurred, ground to a fine powder using a Wig-L-Bug amalgamator (model 3110-3A, Crescent Dental Manufacturing Co. USA). Powdered samples were dried an additional 24 hours at 65 °C, weighed (1.00 +/- 0.10 mg) and packaged into tin capsules.

Fucus

Fucus was collected from three replicate 10 cm^2 quadrats placed one vertical meter below the top of the *Fucus* zone along each transect. Whole plants whose holdfasts were within the quadrat were collected. In the event that a quadrat had no *Fucus,* the nearest adjacent plant was collected. Samples were rinsed with fresh water and any foreign biomass (i.e. small invertebrates, attached algae) removed. All samples were collected within one week in June 2002. Samples were frozen at -20 °C until such time as they could be dried at 55°C until no further changes in mass occurred (1-4 days). Samples were taken from the terminal ends of each plant and ground to a fine powder using a Wig-L-Bug amalgamator (model 3110-3A, Crescent Dental Manufacturing Co. USA). Powdered samples were dried an additional 24 hours at 55°C, weighed (2.66 +/- 0.26 mg) and packaged into tin capsules.

Eelgrass

Eelgrass was collected from seven meadows (each approximately 10 m wide, 30 m long), four within 1400 m of farms, three at reference locations. A total of 30 plants were collected from each meadow in July 2002, 10 from each end and center of each meadow, acting as three replicate samples. Plants were rinsed in fresh water and any foreign biomass (eggs, encrusting invertebrates, attached algae) removed. All samples were collected within a one week period. Samples were frozen at -20 °C until such time as they could be dried at 55°C until no further changes in mass occurred (1-4 days). Samples were taken from the youngest shoots of each plant (to represent the most recent growth) and ground to a fine powder using a Wig-L-Bug amalgamator (model 3110-3A, Crescent Dental Manufacturing Co. USA). Powdered samples were dried an additional 24 hours at 55 °C, weighed (1.68 +/- 0.16 mg) and packaged into tin capsules.

Mussels

Mussels were collected from three sources; four farms, the intertidal zones adjacent to those farms, and four reference locations in July 2003. Mussels from the farm structures were collected by hand approximately one meter below the surface of the water, as close to the center of the farm as possible. Mussels from intertidal zones were collected from the lower end of the mussel band to match submergence time (and thus potential feeding time) as closely as possible with the farm structure mussels. Mussel shells were cleaned to remove any foreign biomass, then frozen at -20°C until they could be dried at 55°C until no further weight changes occurred (2-4 days). Soft tissue was removed from 10 individuals (approximately 10 mm in length) from each sample and ground to a fine powder using a Wig-L-Bug amalgamator (model 3110-3A, Crescent Dental Manufacturing Co. USA). Powdered samples were dried an additional 24 hours at 55 °C, weighed (1.0 +/- 0.1 mg) and packaged into tin capsules. Three subsamples from each sample were prepared.

3) Sample Analysis

Mass spectrometry analysis for all samples was performed at the Stable Isotope Facility, University of Saskatchewan, Saskatoon, Canada using a Europa Scientific ANCA NT gas/solid/liquid preparation module coupled to a Europa Scientific Tracer/ 20 mass spectrometer. Isotopic contents are expressed in "delta" (δ) notation representing the difference between the isotopic content of the sample and known standards (atmospheric N_2 for nitrogen) This is expressed in parts per thousand (‰) calculated by the following formula:

$\delta^{15}N = (R_{sample}/ R_{standard} - 1) \times 1000$, where R is the ratio of the heavy isotope (^{15}N) to the light isotope (^{14}N). (eqn.1)

4) Data Analysis

In order to determine the effect of treatment (farm/no farm) and the spatial scale(s) most responsible for variation in the *Fucus* and eelgrass data, I used a mixed model nested ANOVA (SPSS version 10.07, 2000) with treatments (farm/no farm) as fixed factors, locations, sampling stations (*Fucus* only) as random factors. I performed variance components analyses to quantify (as %) variation contributed from each spatial scale. For mussels, a paired samples t-test was performed comparing mussels collected from farm structures and adjacent intertidal zones, because samples collected from within the same location are not independent and location has been shown to be a main source of variation for all variables under investigation in this study. Additionally, I used one-way ANOVA with source of mussels (farm structure, farm intertidal zone, reference intertidal zone) as fixed factors and locations as replicates. When a factor was significant, Student-Newman-Keuls post-hoc tests were performed to identify differences. The ANOVA assumption of normality of residuals was checked using Q-Q plots, transformations were not required. When ANOVA found significant differences among groups, but the assumption of homogeneity of variance of residuals was violated (Levene's test), Kruskall-Wallace non-parametric tests were preformed. In every case, Kruskall-Wallace tests did not change the significance of ANOVA.

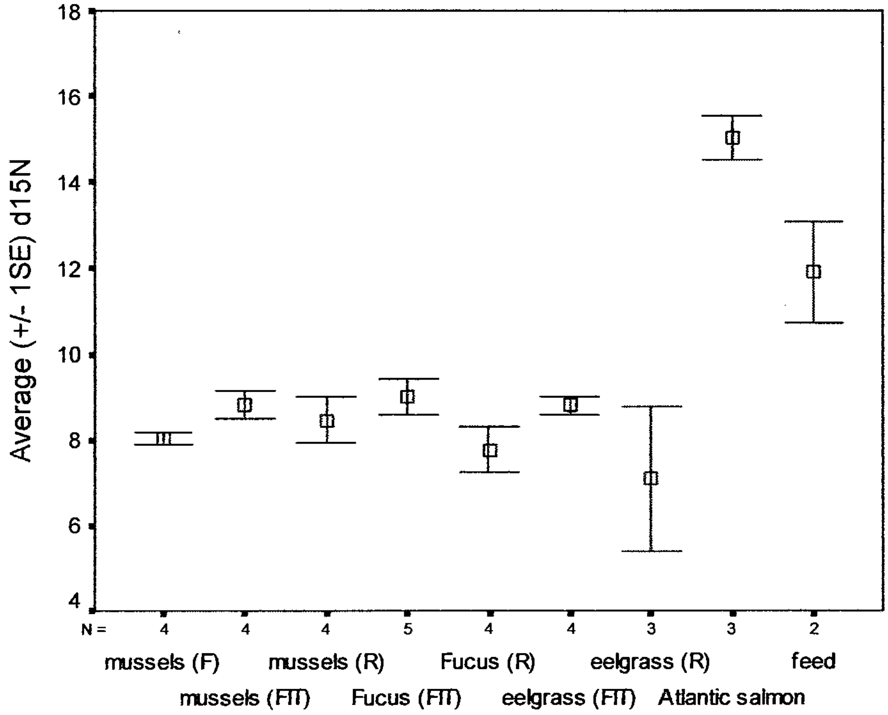
5) Power Analysis

To investigate the possibility of type II error (failing to reject the null hypothesis when it is false) I performed a post-hoc power analysis for treatment effects. I determined the *minimum* significant effect size detectable given my sample size, and calculated the *minimum* sample size required for the observed effect size to be significant (Zar 1999).

Results

Average $\delta^{15}N$ values for salmon farm wastes and putative consumers are shown in Figure 2. Atlantic salmon muscle tissue ($\delta^{15}N$ 14.0-15.25 °/₀₀) and pelleted feed ($\delta^{15}N$ 10.8-13.1°/₀₀) were significantly more enriched for ^{15}N than the primary producers and consumers by 3-7°/₀₀ (one-way ANOVA, $F_{(8,24)}= 12.81$, $p= 0.00$, Figure 2). The isotopic composition of primary consumers and producers were not different from one another, occupying the same range of 6-10°/₀₀. Although not significant, *Fucus*, eelgrass and mussels growing near farms were on average ^{15}N enriched relative to reference locations (Figure 2). Samples of *Fucus* and eelgrass were often more isotopically enriched than mussels (Figure 2).

Figure 2. Average (+/- 1 SE) $\delta^{15}N$ (°/∞) of mussels, *Fucus*, eelgrass and salmon farm wastes, collected from farm intertidal zones (FIT), reference locations (R), and farm structures (F). Atlantic salmon are represented by muscle tissue rather than actual metabolic waste, and slightly overestimate $\delta^{15}N$.



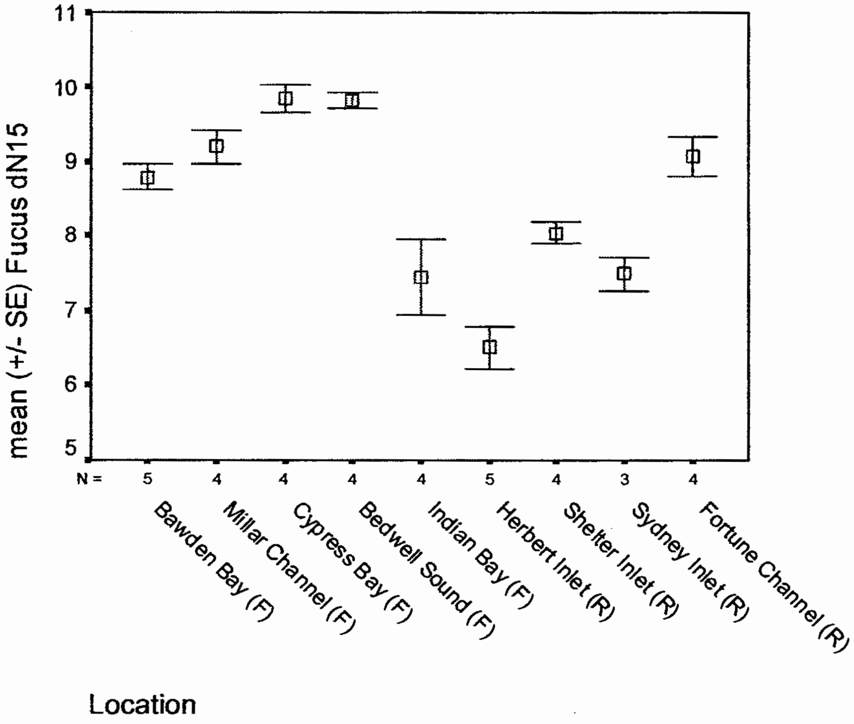
Fucus

Fucus collected from farms was on average slightly enriched for ^{15}N in comparison to reference locations (Figure 2), but this difference was not significant. Treatment was not a significant source of overall variation (p= 0.116 Table 1). Average $\delta^{15}N$ values for farms and reference locations ranged 7.46-9.85 °/₀₀ and 6.51-9.08 °/₀₀ respectively (Figure 3). There was significant variation among locations (Table 1) and the three highest ranking locations were farms (Figure 3). Examination for effect of distance from farm (100's of meters) within each location did not reveal the predicted gradient. Differences among sampling stations were significant at two farms, Cypress Bay and Tofino Inlet, but did not indicate that distance from the farm had any effect on isotopic composition of *Fucus*. Sampling stations with the highest *Fucus* biomass also had the highest $\delta^{15}N$ values at Bawden Bay, Bedwell Sound, Fortune Channel, Herbert Inlet and Sydney Inlet. This correlation was not consistent across all sampling stations at these locations. Location and treatment were the primary sources of variation, 51 % and 27 % respectively (Table 1). Variation within locations and among transects had small contributions to overall variation, 10 % and 12% respectively.

Table 1. Evaluation of the effects of treatment (farm/no farm), location, and distance from farm (sampling station) on $\delta^{15}N$ of *Fucus* using nested ANOVA and variance components analysis (sampling station nested within location, location nested within treatment). Replicate samples were taken from three transects at each sampling station for *Fucus*. Three replicate samples of eelgrass were collected from four meadows near farms, and three at reference locations. Each effect above transect (sampling station, location, treatment) was tested by using the MS of the next lowest factor as the error term. For both *Fucus* and eelgrass, location describes most of the variation.

Variable	Source of variation	df	MS	F	p	Variance component
Fucus	Treatment	1	41.91	3.219	0.116	27%
	Treatment(location)	7	13.12	15.9	0.000	51%
	location(sampling station)	28	0.829	3.04	0.000	10%
	residual (transect)	74	0.243			12%
Eelgrass	Treatment	1	15.54	1.47	0.28	12%
	Treatment(location)	5	10.54	57.34	0.00	83%
	residual	14	0.184			5%

Figure 3. Mean (+/-SE) δ^{15}N (‰) of *Fucus* at five farms (F) and four reference (R) locations in Clayoquot Sound 2002. Each location is represented by samples from 3-5 sampling stations spaced hundreds of meters apart.



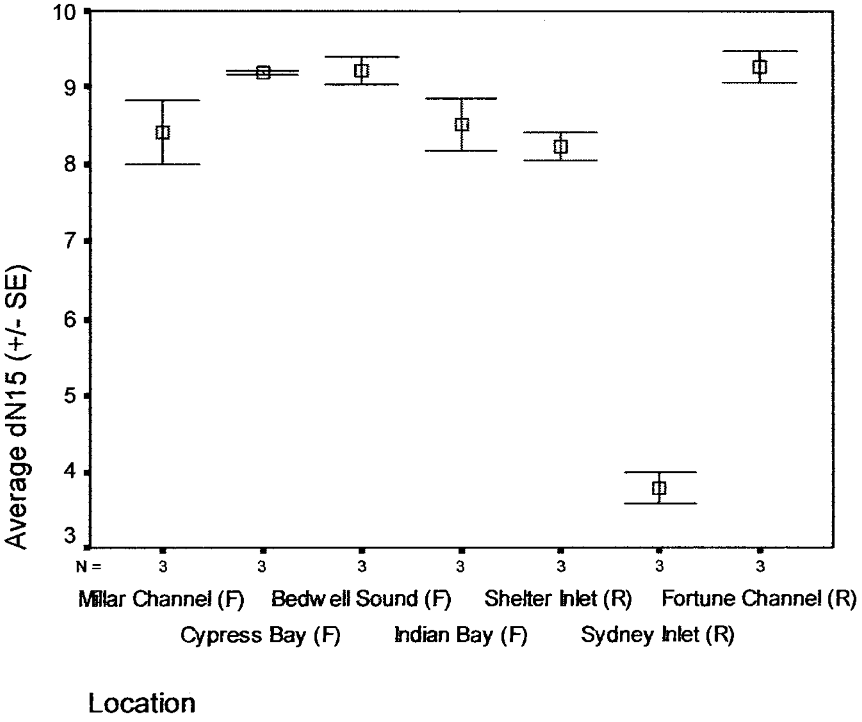
Eelgrass

Eelgrass collected from farms was on average slightly enriched for ^{15}N in comparison to reference locations (Figure 2), but this difference was not significant. Treatment was not significant source of overall variation and explained 12% of the overall variation (p= 0.28 Table 1). Average δ^{15}N values for farms and reference locations ranged 8.40-9.21 ‰ and 3.78-9.28 ‰ respectively (Figure 4). Ranking locations by d^{15}N values showed little variability among locations (Figure 4). However, location was a significant source of variation (p= 0.00), and described 83% of the overall variation (Table 1). Sydney Inlet was the only location significantly different from the others (Figure 4). Examination for the effect of distance of meadows from farms revealed no relationship (Pearson's correlation =0.687, p= 0.313) (Table 2). Eelgrass ^{15}N values were similar to that of *Fucus* (Figure 1), except in Sydney Inlet (3.78 °/₀₀ eelgrass, 7.5 °/₀₀ *Fucus*), possibly identifying the baseline δ^{15}N of the food web in Clayoquot Sound.

Table 2. Distance of eelgrass meadows from salmon farms and their corresponding δ^{15}N values. Pearson's correlation revealed no relationship (r= 0.687, p= 0.313).

Location	Distance (m)	Δ^{15}N (°/₀₀)
Indian Bay	206	8.50
Millar Channel	465	8.40
Cypress Bay	527	9.19
Bedwell Sound	1368	9.21

Figure 4. Average (+/- SE) δ^{15}N (‰) for eelgrass meadows growing near four salmon farms (F) and three reference (R) locations throughout Clayoquot Sound, 2002. Each location is represented by three samples taken from the center and each end of each meadow.



Mussels

Mussel δ^{15}N was not significantly affected by source of sample collection (one-way ANOVA, $F_{(2,9)}$= 1.072, p= 0.382). Mussels growing in intertidal zones adjacent to farms were the most enriched, followed by reference locations, then mussels from farm structures (Figure 5). Mussels collected from farm structures had consistently lower d^{15}N values in comparison to adjacent intertidal mussels (Figure 6), but this difference was not significant (paired samples t-test, t= -2.5 , df= 3, p= 0.088). Mussel δ^{15}N values were similar to published values (8.7 Minagawa and Wada 1984)

Figure 5. Average (+/- SE) $\delta^{15}N$ (°/₀₀) of mussels collected from three sources; four farm structures and their adjacent intertidal zones, and four reference locations. Contrary to predictions, mussels collected from the farm structures are the most depleted for^{15}N.

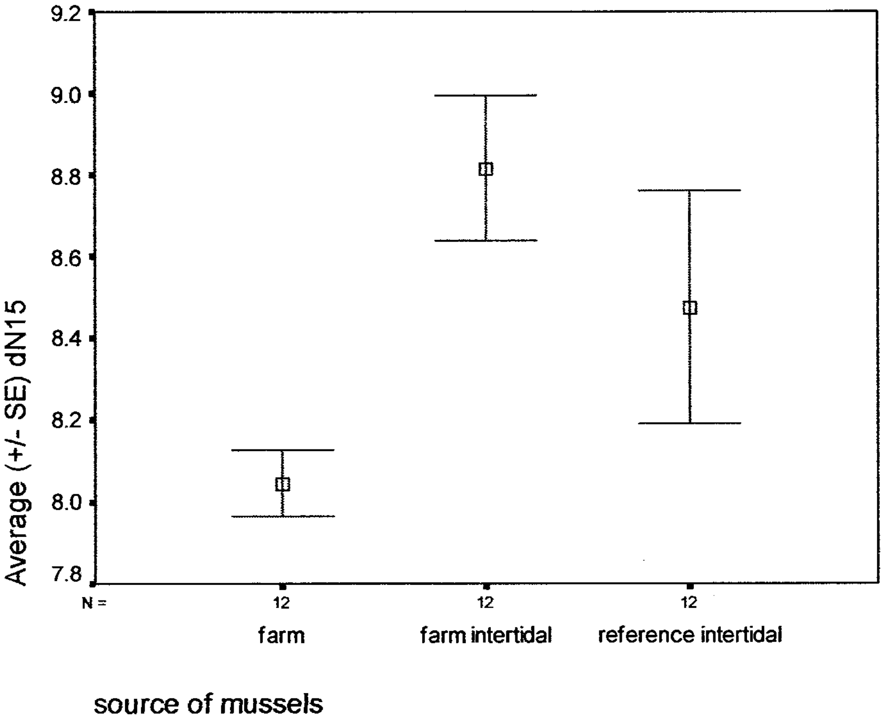
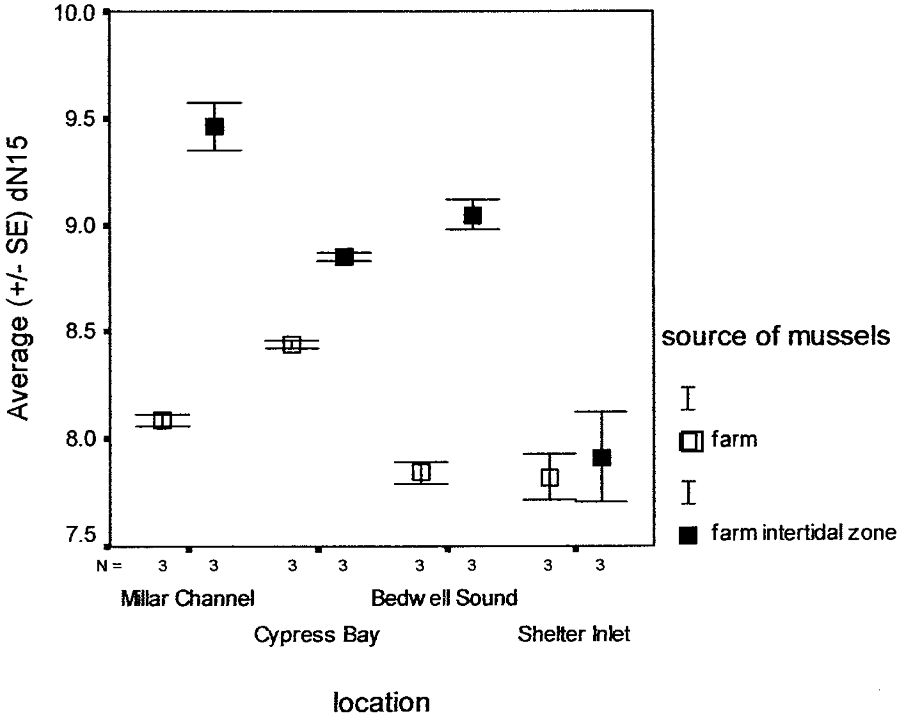


Figure 6. Comparison of average (+/- 1SE) $\delta^{15}N$ (°/₀₀) values for mussels collected from four farms and the intertidal zones immediately adjacent. Note that in all cases, mussels are enriched for ^{15}N in the intertidal zone relative to those growing on the farm, although this relationship is not significant (paired samples t-test, t= -2.5 , df= 3, p= 0.088)



Power Analysis

Table 3 summarizes the results of the post-hoc power analysis for farm/no farm treatment. Effect sizes would need to be 1.7- 6x larger in order to be significant for the given sample sizes. The sample sizes required for mussels and eelgrass are not feasible as there are only approximately 20 active farms at any given time in Clayoquot Sound, and only four farms with eelgrass meadows nearby.

Table 3. Post-hoc power analysis for the effect of treatment (farm/no farm) on ^{15}N content of *Fucus*, eelgrass and mussels throughout Clayoquot Sound. Minimum significant effect size and minimum N required for observed effect size to be significant are reported.

Variable	N	Observed effect size	Min. sig. effect size	Min N for sig. obs. effect Size
Fucus	5 farms 4 reference	1.29 ‰	2.30 ‰	10
eelgrass	4 farms 3 reference	1.82 ‰	5.32 ‰	18
mussels	4 farms 4 reference	0.35 ‰	2.11 ‰	103

Discussion

In this study, the stable isotope ^{15}N does not provide conclusive evidence that salmon farms wastes are used by intertidal organisms. There is no significant enrichment of ^{15}N near farms relative to reference locations, and no effect of distance from farm for any of the organisms investigated. Consumers should theoretically be enriched for ^{15}N in comparison to their resources (DeNiro and Epstein 1980, Wada 1980, Owens 1987, Peterson and Fry 1987). However *Fucus*, eelgrass and mussels growing near salmon farms in Clayoquot Sound were significantly depleted for ^{15}N relative to salmon farm wastes (Figure 2). This indicates that if salmon farm wastes are used by the intertidal organisms in this study, the subsidy is minor and any associated isotopic enrichment is not detectable above natural variation. This questions whether salmon farm wastes are available to intertidal organisms, and the usefulness of variation in $\delta^{15}N$ for tracing the flow and fate of (at most) small subsidies of salmon farm wastes.

Inaccessibility of salmon farm wastes to intertidal organisms becomes more evident when farms are investigated individually. Variation within each location describes only 10% of variation for *Fucus*. Data from a pilot study (following the nested design at two farms and three reference locations) shows that variation within locations explains 13% of variation in mussel $\delta^{15}N$. This indicates that there is no effect of distance to a farm on isotopic composition of *Fucus* and mussels. Particulate matter originating from the farm may be intercepted by mussels growing on the farm. Integrating the culture of filter feeders (e.g. oysters or mussels) with salmon has been examined as a candidate solution to reducing the amount of particulate wastes released to the surrounding environment (Troell and Nordberg 1998, Troell et al. 1999). Other researchers have suggested that mussels growing on salmon farms prevent phytoplankton from surpassing ambient levels (Taylor *et al.* 1992) by virtue of their ability (in high densities) to significantly deplete particulate matter from the water column (Cloern 1982, Wildish and Kristmanson 1984, Frechette and Bourget 1985a,b, Asmus and Asmus 1991). In this study, mussels sampled directly from farm structures showed no evidence of usage of salmon farm wastes. However, this could be because the mussels sampled were approximately 1m from the surface of the water and not deep enough to actually intercept any farm derived particulate matter. Mussels growing at greater depths would be more likely to intercept farm derived particulates. Additionally, it has been demonstrated that transport of solid particulates is largely vertical and within 60m (variable depending upon local current speeds and directions and depth) of the edges of the cages (Brown *et al.* 1987, Kaspar *et al.* 1988, Sutherland *et al.* 2001, Cheshuk *et al.* 2003) thus significantly reducing the availability of solid wastes to suspension feeders in the intertidal zones of Clayoquot Sound. One farm was 52m from the intertidal zone, the others ranging 170-400m, thus close to, or outside reported affected zones. Uneaten pellets are known to fall to the seafloor largely intact, thus not contributing to suspended particulate matter (Cheshuk *et al.* 2003). Currents may be flushing dissolved and particulate wastes from the farms in directions away from the intertidal zone. Currents can alter the density and distribution of dissolved and particulate nutrients by dispersion (Mackas *et al.* 1985). This possibility combined with the distance the farm is from shore could interact to dilute salmon farm wastes making them undetectable above natural levels. Additional dilution of salmon farm wastes is facilitated by pulsed feeding (and release of metabolic wastes) of fish, but continuous feeding by mussels and nutrient uptake by *Fucus* and eelgrass.

The usefulness of variation in $\delta^{15}N$ as an indicator of nitrogen sources is based upon the assumption that sources must have unique and detectably different $\delta^{15}N$ compositions from one another and from their consumers (Owens 1987). Farm wastes were significantly enriched for ^{15}N relative to eelgrass, *Fucus* and mussels, thus meeting this assumption. However, an organism's isotopic content is also a function of resource preference, resource availability, and the relative contribution of each resource to the organisms diet (DeNiro and Epstein 1980, Wada 1980, Owens 1987, Peterson and Fry 1987). Large differences in isotopic compositions between resources and consumers will have a larger effect on the consumer's isotopic composition. If salmon farm wastes are only marginally available to intertidal organisms, then the overall effect on the isotopic composition of the organisms utilizing them will be small.

In BC, salmon farms release an average of 740kg waste/day (Naylor *et al..* 1998, BC Salmon Farmers Association). The instantaneous concentration of waste surrounding the farms (volume roughly calculated as $\pi r^2 h$, where radius= distance to nearest sampling station, h= depth below farm, and assuming even distribution of waste throughout this volume) ranged between 19 and 4355 μg/L. Accounting for settling of solids (Sutherland *et al.* 2001, Cheshuk *et al.* 2003) and distance, if I assume only 0.1% of total wastes released are transported to the intertidal zone, this translates to between 0.019 and 4.3 μg/l of additional resources. Average phytoplankton biomass at each location ranged 0.5-12 μg/L (data from Chapter 2 of this study). Theoretically, the proportional addition of wastes ranges 1-453%, but mostly below 20%. The largest theoretical contribution would be at Tofino Inlet, as it was the closest to shore (52m) and phytoplankton biomass was consistently below 2 μg/L. Tofino Inlet would be the most likely farm to supply the intertidal zone with a resource subsidy, however, no farm effects were detected for any variables. Likely explanations are currents flushing wastes away, and below average annual production.

The three focal species, which span two trophic levels, have similar average $\delta^{15}N$ and occupy the same range of stable isotope content, approximately +6-10 ‰ (Figure 2). Isotopic fractionation theory predicts that ^{15}N should be on average be 3-5‰ enriched between successive trophic levels (DeNiro and Epstein 1981, Miagawa and Wada 1984, Owens 1987). This implies that *Fucus* and eelgrass (most likely as detritus) are not significant dietary items of intertidal mussels, and that isotopic variation in primary producers can be important when identifying trophic relationships in food webs (Cabana and Rasmussen1996, VanderZanden and Rasmussen 1999). Mussels rely on phytoplankton as their primary food source during the summer months (Seed 1969, Widdows *et al.* 1979), which is likely isotopically distinct from eelgrass and *Fucus* due to differences in nutrient preference, source and uptake rate (Pedersen and Borum 1996).

Eelgrass, *Fucus* and phytoplankton utilize NH_4 and NO_3. However, phytoplankton uses NO_3 preferentially (Owens 1987, Yin *et al.* 1991) and the nitrogen requirement for equivalent biomass and time by fast-growing phytoplankton can surpass that of *Fucus* by 30x (Pedersen and Borum 1996). Phytoplankton assimilating NO_3 are generally significantly depleted in ^{15}N than in comparison to when assimilating NH_4 (Wada and Hattori 1976). Phytoplankton blooms could reduce the availability of NO_3 to *Fucus* by virtue of their rapid uptake of it. In addition, phytoplankton should theoretically prefer the isotopically lighter forms of NO_3 and NH_4 in order to maximize their growth potential. However, other species of fast-growing green algae have been shown to not have any isotopic preference for NO_3 or NH_4 (Cohen and Fong 2004). These factors taken together could be leaving an isotopically enriched nitrogen pool for the slower growing eelgrass and *Fucus*, and thus producing variation in the isotopic baseline of primary producers.

Stable isotope content was variable among locations for each species. Differences among resources for stable isotope content, coupled with resource availability and consumer preference leads to variable isotopic content in consumers.

Primary producers use both dissolved NO_3 and NH_4. NH_4 is generally enriched for ^{15}N relative to NO_3. The relative concentrations of dissolved NO_3 and NH_4 available to primary producers are variable based on oxygen content of the water (Owens 1987), supply of upwelled water (Mackas *et al.* 1980, Yin *et al.* 1996), input of fresh water (riverine or rain) (Mackas *et al.* 1980, Valiela *et al.* 1992, Page *et al.* 1995) and season (Mackas *et al.* 1980, Daly and Smith 1993, Yin *et al.* 1996). These factors likely contribute to spatial variation in $\delta^{15}N$ of primary producers throughout Clayoquot Sound, as it has rivers of varying flow capacity, is situated next to an upwelling zone to which the various inlets and channels are variably exposed, and samples were collected in different years.

Preference for NO_3 or NH_4 varies among primary producers, with availability and environmental conditions (Owens 1987, Cohen and Fong 2004). *Fucus* uses both NO_3 and NH_4 (Topinka and Robbins 1976, Wallentinus 1984, Thomas *et al.* 1987), however the presence of NH_4 inhibits NO_3 uptake (Thomas *et al.* 1985). At low salinities however, for instance from rainfall or river runoff, *Fucus* increases NO_3 uptake by up to 50% (Reuter and Robinson 1986). Thus *Fucus* has the potential to be variable in isotopic composition, which appears to be the case throughout Clayoquot Sound.

The $\delta^{15}N$ of eelgrass would likely vary as a function of NH_4 availability. Eelgrass can assimilate NH_4 from sediment water, which could tend to be ^{15}N enriched as eelgrass meadows tend to be sites of sediment deposition and nutrient cycling through decomposition (Short 1987). Competition from fast growing phytoplankton and epiphytes can reduce the availability of NH_4 to eelgrass (Short 1987, Burkholder *et al.* 1992).

Spatial variation in mussel $\delta^{15}N$ is likely due to their variable diet (Seed 1969, Widdows *et al.* 1979). Dietary items include phytoplankton, zooplankton, organic detritus and solid wastes of intertidal organisms (Seed 1969, Widdows *et al.* 1979, Mazzola and Sarà 2001). There can be considerable variation in $\delta^{15}N$ within and among each of these resources (DeNiro and Epstein 1980, Owens 1987, Duggins 1989), along with the relative abundance of each in the diet of a mussel thus contributing to spatial variation in mussel $\delta^{15}N$.

Mussels collected from the farm structure are on average more depleted for $\delta^{15}N$ than intertidal mussels, and was a consistent trend at the four farm locations (Figure 6). This difference could be explained by isotopic differences in food sources. Mussels collected from the farm are likely eating mainly phytoplankton, rather than salmon farm wastes. This could be an artifact of the depth at which they were collected, approximately 1m below the surface of the water where wastes are likely in low concentrations. Mussels growing in the intertidal zones are likely to encounter other resources such as kelp detritus (Duggins *et al.* 1989, Bustamente *et al.* 1995), and fecal matter from other intertidal organisms (Mazzola and Sarà 2001) which tend to be isotopically enriched relative to phytoplankton (Wada and Hattori 1976, Peterson and Fry 1987).

The stable isotope ^{15}N has been used successfully to trace the flow and fate of anthropogenic sewage in marine food webs (Rau *et al.* 1981, Risk and Erdmann 2000). In contrast, the few studies that have investigated the effect of aquaculture wastes on marine food webs using stable isotopes have focused largely on the use of ^{13}C. A recent study by Vizzini and Mazzola (2004) using ^{13}C and ^{15}N found significant ^{15}N enrichment in particulate organic matter, macroalgae, seagrass, bivalves, other benthic invertebrates and fish, directly adjacent a land-based salmon farm waste outfall and up to 500m away. These results contrast those of this study likely for two reasons. The mechanism of waste deposition to the ocean in the study by Vizzini and Mazzola (2004) is from an outfall pipe, which results in a concentration of wastes near the mouth of the outfall.

Wastes from net cages are released from a larger area (i.e. the area of the nets) and are likely significantly more dilute even at the point of release. Differences between the concentrations of wastes at the sources and at sampling stations 500m away would likely be much larger for the system in Vizzini and Mazzola's study. Secondly, the distance between the source and the closest sampling points are different. Vizzini and Mazzola collected samples at the outfall source, whereas the closest samples were collected 50-465m away from the source in the present study. Again, this difference could affect the magnitude waste concentration, since dispersal mechanisms such as currents are likely a factor.

^{15}N was not a useful tracer of salmon farm derived nitrogen into the adjacent intertidal food web in this study. Dilution and dispersion of wastes by currents, distance of farm from shore and high natural isotopic variability in sources and consumers combine to result in no detectable enrichment in *Fucus*, eelgrass or mussels in Clayoquot Sound. In addition, the lack of isotopic information of other sources (phytoplankton, seawater and suspended particulate matter) prevent confident assessments of trophic relationships in this study.

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Chapter 4

Comparison of intertidal invertebrate community structure among areas of Clayoquot Sound, British Columbia, with and without salmon net-cage farms.

Keywords: intertidal community structure, bottom-up forces, salmon farm wastes, resource subsidy, productivity gradients.

Abstract

Intertidal community structure is shaped by physical and biological factors. The least well understood is the influence of nutrient resources, as they are difficult to manipulate experimentally *in situ* and over large spatial scales. Intertidal invertebrate community structure was compared among areas of Clayoquot Sound BC, with and without salmon farms to investigate the effects of a putative resource subsidy. Farms had no effect on community composition, species richness, predator and total species abundance on large (kms) or small (100's of meters) scales. Natural variation and minimal availability of salmon farm wastes to adjacent intertidal zones likely account for these results. Species richness and predator abundance were positively correlated with variation in *Fucus* standing in accordance with current species richness-productivity theories, and trophic-dynamic models.

Introduction

Historically, intertidal community structure was thought to be shaped by physical factors such as wave force (Dayton 1971, Menge and Sutherland 1976), physical transport (Gaines and Roughgarden 1985), and top-down biological forces such as predation and competition (Connell 1961, Paine 1973, Menge and Sutherland 1976, Lubchenco 1978, Menge 1978). Nutrient quantity and quality were largely ignored as determinants of community structure because they were thought not to vary appreciably over the typically small (<1km) spatial scales encompassed by the majority of studies (Menge 1992, 2000), and because of the logistic difficulty in manipulating productivity in the open ocean (Menge 1992, Abrams 1993, Menge *et al.* 1997*a*). Recently however, on larger spatial scales (>1km) it has been noted that variation in intertidal community structure could not be explained entirely by physical or top-down biological factors (Bustamante *et al.* 1995*a*, Menge *et al.* 1997*a,b*, Nielsen 2001), and that perhaps bottom-up factors such as variability in nutrients and primary productivity might provide additional explanatory power.

Intuitively it follows that productivity of plants will affect community biomass, since plants fix solar radiation into the complex organic substances that provide the basis for all life (Lindemann 1947). Hunter and Price (1992) point out that although removal of consumers has the potential to modify the system (e.g. Paine 1966, 1974, Estes *et al.* 1978, Carpenter *et al.* 1987, Wootton and Power 1993), the removal of plants leaves no system at all.

The distribution of biomass within and among trophic levels is highly variable, and depends largely on species interactions, life history characteristics and trophic structure of the community (Hairston *et al.* 1960, Fretwell 1977, Oksanen *et al.* 1981, Carpenter *et al.* 1987, Hunter and Price 1992, Abrams 1993, Wootton and Power 1993, Bustamante *et al.* 1995*b*, Polis and Strong 1996, Leibold 1989, 1999). As a result, there are many models and hypotheses predicting the effects of increasing productivity to community structure, even within the same community. The best predictive model can change along productivity gradients (Fretwell 1977, Wootton and Power 1993, Bustamante *et al.* 1995*a*), and with complexity of communities, i.e. reticulate (web-like) vs. chain-like trophic associations (Power 1992, Abrams 1993, Polis and Strong 1996).

Increased productivity can elevate the growth of existing individuals, increase a species' reproductive output (Abrams 1993, Leibold 1996, Bohannan and Lenski 2000), increase community diversity within trophic levels (Powell *et al.* 1991, Leibold 1999, Waide *et al.* 1999, Dodson *et al.* 2000, Mittelbach *et al.* 2001), or by the addition of trophic levels

(Lindemann 1947, Fretwell 1977, Oksanen *et al.* 1981, Wootton and Power 1993). Any of these scenarios can alter the type, strength and outcome of species interactions, and affect population dynamics within and among trophic levels (Menge and Sutherland 1976, Oksanen 1981, Hunter and Price 1992, Abrams 1993, Wootton and Power 1993, Leibold 1989, Bohannan and Lenski 2000).

Species richness is a common measure of community diversity and its relationship to productivity has been examined across community types and spatial scales. The most common productivity-species richness relationship is the unimodal "hump-shaped" curve (Leibold 1988, Waide *et al.* 1999, Dodson *et al.* 2000, Mittelbach *et al.* 2001), which some researchers believe to be the true relationship (Rosenzweig 1971, Tilman 1982). However, positive, negative and U-shaped relationships are common across community type and spatial scales (Waide *et al.* 1999, Mittelbach *et al.* 2001). For the hump-shaped relationship, species richness is assumed to vary positively with resource supply in unproductive environments. Species adapted to low and high levels of productivity will be rare but are assumed to be competitively dominant under these conditions. At moderate levels of productivity, species richness will be highest, possibly due to less extreme abiotic factors at moderate productivity levels, and because competition and predation keep superior competitors from becoming dominant, and (Leibold 1999, Dodson *et al.* 2000).

In nature, the trophic-dynamic model (Elton 1927, Lindemann 1942, Fretwell 1977, Oksanen *et al.* 1981) is a suitable predictor of how community structure and dynamics will respond to changes in productivity. The model predicts that along an increasing gradient of productivity, additional trophic levels will form as community biomass accumulates. This model is most powerful when trophic linkages are simple and chain-like (Wootton and Power 1993, Wootton *et al.* 1996), when a species has sufficient influence over the productivity of their resources (Fretwell 1977, Oksanen *et al.* 1981, Estes *et al.* 1978, 1982) or when large spatial scales are considered (Dodson *et al.* 2000, Post *et al.* 2000). However, theoretical and experimental evidence shows that factors such as species diversity within trophic levels (Abrams 1993, Polis and Strong 1996), spatial scale (Waide *et al.* 1999, Post *et al.* 2000) and environmental heterogeneity (Menge and Sutherland 1976, Abrams 1988, Hunter and Price 1992, Power 1992) produce many more patterns of species interactions, population dynamics and biomass dispersal throughout the community when productivity is varied. Additionally, variation in resource quality (White 1978, Halaj and Wise 2001) or edibility (Lubchenco 1978, Leibold 1989, Bohannan and Lenski 2000), differential exploitative abilities (Bohannan and Lenski 2000) and behavioural changes of consumers (Abrams 1984, Anholt and Werner 1998) can decouple productivity relationships between trophic levels, vary the relative importance of predation and competition and thus the structure and dynamics of populations within the community (Abrams 1993, Leibold 1989, Bohannan and Lenski 2000).

An experimental method for testing the effects of productivity on community structure is to examine a community where resources are subsidized from adjacent communities or systems (Kline *et al.* 1990, 1993, Duggins *et al.* 1989, Folke and Kautsky 1989, Powell *et al.* 1991, Bustamante *et al.* 1995*b*), or along productivity gradients (Power 1992, Bustamante *et al.* 1995*a*, Waide *et al.* 1999). Open net-cage salmon farms offer a unique opportunity to perform replicated, comparative studies on the effect of resource subsidies to the adjacent intertidal community. During this study (2001-2), British Columbia (BC) had 83 operational farms and an average annual production of 67 700 tonnes (range 50,000-85,400 tonnes round wt, BC Salmon Farmers Association), which averages 816 tonnes fish per farm per year, and 270 tonnes of waste per farm per year (based on 1.5 feed conversion ratio, Naylor *et al.* 1998). The dissolved and particulate wastes released from salmon farms (as fish metabolic wastes and excess feed) have been shown to have significant effects on the growth and abundance of a variety of marine organisms that occur intertidally (Wallace 1980, Jones and Iwama 1991, Ahn *et al.* 1998, Mazzola and Sarà 2001, Vizzini and Mazzola 2004), but to date, no studies have investigated the effects of salmon farm wastes on the structure and functioning of intertidal communities (Burd 1995). In addition, the intertidal communities are good candidates for the study of resource subsidies or productivity gradients, because of their typically large spatial extent (Stephenson and Stephenson 1949, Bustamante *et al.* 1995*a,b*, Menge *et al.* 1997*a*), allowing direct comparisons between target areas and adjacent unaffected areas.

From another part of this study, *Fucus* standing biomass was found to be variable among locations (kilometers apart), and these relative differences were used as the basis for a productivity gradient within Clayoquot Sound. *Fucus* standing biomass is useful for comparisons of intertidal primary productivity as it is long-lived, is tolerant of low nutrient levels (Pedersen and Borum 1996) and is unpalatable to grazers when mature (Lubchenco 1983). Thus *Fucus* has the potential to accumulate a wide range of available nutrients into algal biomass across over long time periods with little loss from predation. *Fucus* was also present throughout Clayoquot Sound allowing all locations to be included in the analyses. The drawbacks to using *Fucus* as a measure for accumulated intertidal primary production is that it is only one of several algal species present, and its growth is generally slower than most other species (Wallentinus 1984, Worm and Sommer 2000). *Fucus* is cited as growing best where there is little competition from other organisms (Edelstein and McLachlan 1975, Thom 1983) suggesting that *Fucus* standing biomass would be most affected at high nutrient concentrations when fast-growing green algae would present more competition. Additionally, *Fucus* can be removed by scouring from logs, which would lead to underestimates of standing biomass. Scouring by logs was assumed to be equal throughout Clayoquot Sound.

The intertidal community of Clayoquot Sound consists of several trophic guilds, which are groups of organisms that utilize similar resources (Menge and Sutherland 1976): producers (phytoplankton, kelps and macrophytes), omnivores (mussels and barnacles), strict herbivores (limpets, periwinkles), strict carnivores (whelks, seastars, crabs). The dissolved wastes from salmon farms are a resource subsidy to producers, which in turn are resources for herbivores. Particulate wastes are a resource subsidy for filter feeders and zooplankton. Zooplankton are also a principle food source for intertidal filter feeders.

Several aspects of intertidal community structure were investigated in response to a putative resource subsidy from salmon farms, with the following predictions:

1) Communities adjacent to salmon farms would be different than those distant from the farm, community *similarity* would increase with distance from the farm, as the farm derived subsidy becomes unavailable. The overall abundance of individuals was predicted to increase with proximity to the farm, as was species richness.

2) Elevation of productivity due to a resource subsidy from salmon farms would result in the addition of a predator guild or an increase in the abundance of predators (in those areas where they already occur), increased species richness and total abundance of individuals.

3) Following an increasing productivity gradient represented by *Fucus* standing biomass, species richness, total abundance of individuals and predators would also increase.

Methods

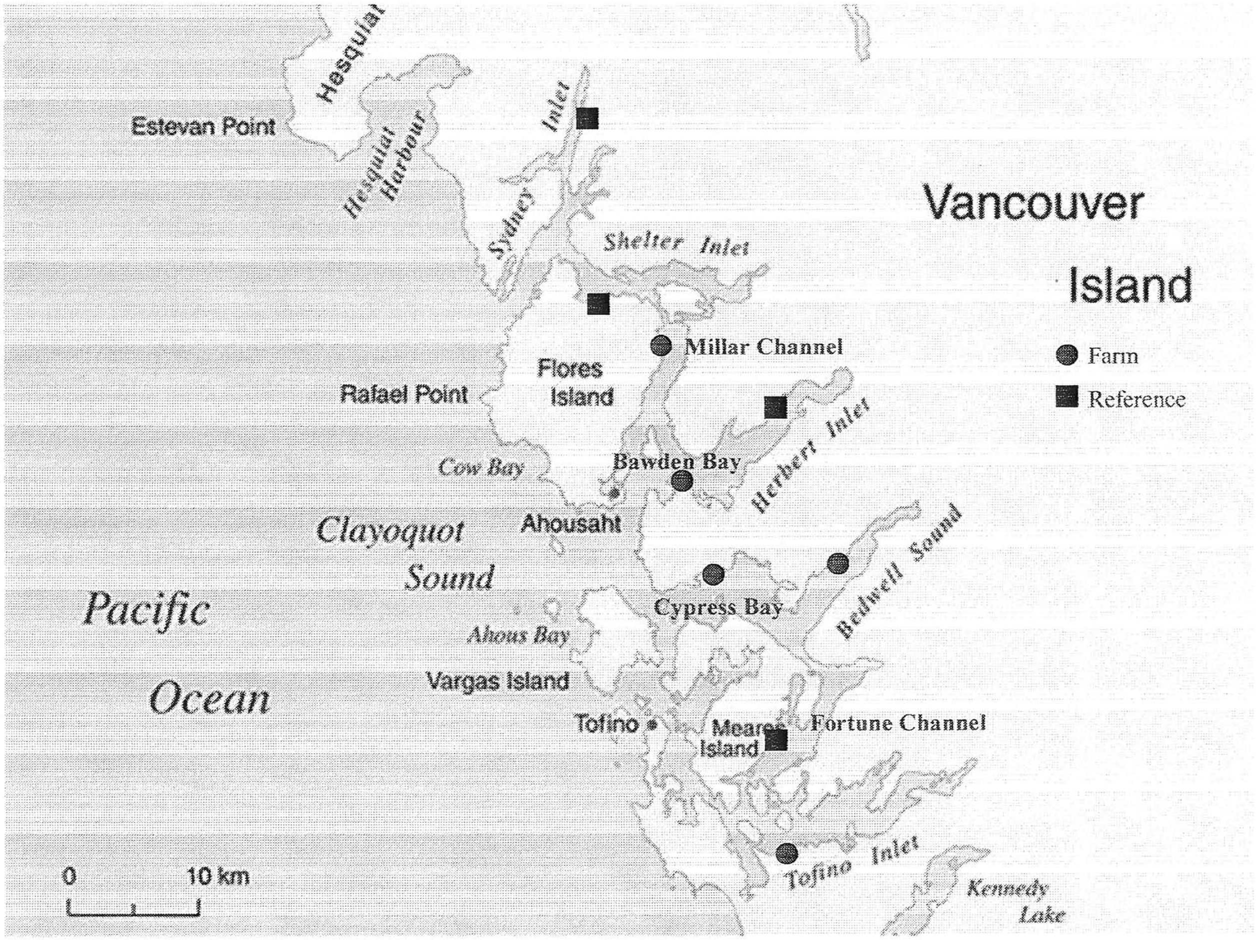
1) General Sampling Design

This study was conducted in the *Fucus* band of the mid-intertidal zone, adjacent to five salmon farms and four reference locations in Clayoquot Sound, British Columbia during May-September 2002 (Figure 1).

I employed a three-level nested sampling design to determine the spatial scale at which ecological differences may be detected; locations (kms apart), sampling stations nested within locations (100s of m apart), and transects nested within sampling stations (10s of m apart). Locations were designated as treatments where there were farms, or references where there were no farms. Reference locations were meant to represent the natural ecological conditions (including variation) of Clayoquot Sound. Each location was assigned between three and six sampling stations, spaced approximately 200-2300 meters apart (figs of each location). All farms had 4-6 sampling stations. At farm locations, one sampling station was placed directly adjacent to the farm (50- 465m away), 2-4 others spaced approximately 200-800 meters apart up and downstream from the farm. The final sampling station was positioned 900-7000 meters away from the most distal sampling station to act as a within-location reference. Preliminary results from a pilot study indicated that reference stations should be located within the same inlet or channel as the farm in order to account for interlocation variation, which was usually the most significant source of variation. The same design was replicated at reference locations. The spacing of sampling stations is not perfectly replicated at all locations but the design is conserved. Sampling stations were chosen based upon accessibility to the researcher, similar slope and bearing; most often on small bedrock headlands.

Three transects were established at each sampling station. Transects were spaced a few meters apart perpendicular to the waterline, their positions based on maintaining similar slope and bearing.

Figure 1. Location of study sights (Farm and Reference Locations) in Clayoquot Sound.



Sampling Protocol

Community data were obtained from 0.5 m^2 quadrats positioned one vertical meter below the top of the *Fucus* band along each transect. All mobile and sessile macroinvertebrates were identified to species and counted. This method underestimates total species richness because small species (<1mm) are missed, but the procedure provides comparable information. Mussel and barnacle abundances were typically very high and thus estimated from three replicate 5 cm^2 subquadrats within the 0.5m^2 main quadrat. Subquadrats were positioned in each of the top corners and one at the bottom center for mussels, one each in the bottom corners and one at the top center for barnacles. This procedure was to ensure independent samples for all species. All data were collected over four weeks in June and July 2002 .

Statistical Analysis

In order to determine if community structure was affected by proximity to a salmon farm, I tested the hypothesis that sampling stations would be more similar if they were farther away than 400m from a salmon farm (400m is the farthest distance any farm is from the intertidal zone). The sampling station closest to the farm was also compared against all other sampling stations. For all locations, I tested the hypothesis that sampling stations closer together would share more similar communities than with those further apart. I used Bray-Curtis dissimilarity matrices (Krebs 1989) and distance matrices for each farm location to quantify spatial variation in community structure. The Bray-Curtis index quantifies the difference between two communities using species composition and abundance and expresses the difference as a proportion of the maximum possible difference (Krebs 1989). Completely dissimilar communities have a value of 1, completely similar communities have a value of 0. The first matrix is the Bray-Curtis dissimilarity index, where element $C_{1,2}$ is the dissimilarity coefficient between sampling stations 1 and 2. The second matrix is of spatial distances, where $D_{1,2}$ is the spatial distance (in meters) between sampling station 1 and 2. The 2 matrices are then compared using analysis of similarity (ANOSIM software program R v.1.8.1), a modified version of the Mantel test (Mantel 1967). A correlation coefficient, R_0, is calculated for the original matrices. Since dissimilarities are not independent of each other, ANOSIM employs a randomization test rather than usual significance tests. One of the matrices is randomized numerous times, and correlation coefficients are recalculated for each randomization. If the original matrices had been correlated, the randomizations should have reduced the correlation coefficients. Our measure of significance is the number of times that the original correlation coefficient (R_0) was exceeded by the permuted values. For example, if after 999 permutations and only 1 of the permutated coefficients exceeded R_0 this would give use a significance of 0.002 (one for the original observations plus one permuted coefficient exceeding R_0).

The effect of the farm treatment on species richness was evaluated using an independent samples t-test, using farms as independent replicates. Species richness at each location represents a running total of all species encountered from all sampling stations.

Total species abundances (i.e. total individuals across all species) were analysed using a three-level, mixed model nested ANOVA (SPSS version 10.07, 2000) with treatments (farm/no farm) as fixed factors, locations and sampling stations as random factors; sampling stations nested within locations, locations nested within treatment. I performed variance components analyses to quantify (percent) variation contributed from each spatial scale.

Predators tended to be very low in abundance when present, but their presence in a community can have profound effects even at low densities (e.g. Paine 1974, Estes *et al.* 1978). Since Bray-Curtis is less sensitive to changes in the abundances of rarer species (Krebs 1989), I compared the abundance of predators at locations with and without farms using an independent samples t-test, using locations as independent replicates. Predator abundance at each location was an average of predator abundance at each sampling station.

In order to investigate current food web hypotheses, I looked for relationships between *Fucus* standing crop (determined in another part of this study) and average total species abundance, mussel biomass and predator abundance (trophic-dynamic hypothesis), species richness (productivity-richness hypothesis), using Pearson's Correlation. *Fucus* biomass was used as a surrogate for primary productivity, since it was present at all locations, and is relatively ungrazed at maturity (Lubchenco 1983), and has been shown to increase biomass as limiting nutrients are elevated (Topinka and Robbins 1976, Ronnberg *et al.* 1992). Additionally, there was some variation among locations for *Fucus* biomass and so could represent a productivity gradient at this spatial scale. I used data pooled for each location, because location was the main source of variation for both *Fucus* biomass and total species abundance, with very little variation within locations.

Results

Within each farm location, there was no evidence that the composition of communities from sampling stations classified as near (<400 m away), or far (>400m away) from the farm differed in any consistent way (p = 0.45). Comparing sampling stations closest to the farm with all other sampling stations within a location also showed no difference (p = 0.51). Excluding the most distal sampling station (i.e. the within location reference station) from each analysis did not resolve any differences (p = 0.58). Within a farm location, sampling stations that were closer together were more similar than would be expected by chance (p= 0.03). Within location reference sites were distant from farms and may have a large influence on the result. Excluding these sites eliminates the significant relationship. I expanded this analysis to include all 9 locations as a stratification factor. In this case the matrix correlation was near the level of significance (P = 0.06), indicating that communities in close proximity are more similar than communities far apart.

The presence of a farm had no effect on total species abundance (p= 0.61, Table 1), species richness (t= 0.737, df= 7, p= 0.484, Fig. 2), or predator abundance (t= 1.266, df=7, p= 0.246, Table 2). There was variation among locations for species abundance (p= 0.000, 47% Table 1, Fig. 3), richness (Fig. 2) and predator abundance (Table 2). Four locations (two farms, two references) lacked predators entirely (Table 2).
Differences within locations for species abundance was significant (p= 0.05) but accounted for only 9% of total variation (Table 1), indicating that distance from farms had little effect. Variation among samples accounted for 44% of variation in total species abundance.

Species richness was positively and significantly correlated with *Fucus* production (Pearson's correlation= 0.822, p= 0.007), as was average predator abundance (Pearson's correlation= 0.792, p= 0.011). Predators were present in locations where *Fucus* biomass was as low as 2.16g/ 10cm^2. Average total species abundance was not correlated with *Fucus* biomass (Pearson's correlation= 0.000, p= 1.000).

Fig. 2. Species richness of mid-intertidal macroinvertebrate species at five farms (F) and four reference (R) locations in Clayoquot Sound, 2002. The number of species present in locations with salmon farms was not significantly different from reference locations (t= 0.737, df= 7, p= 0.484). Species richness at each location represents a running total of all species encountered from all samples.

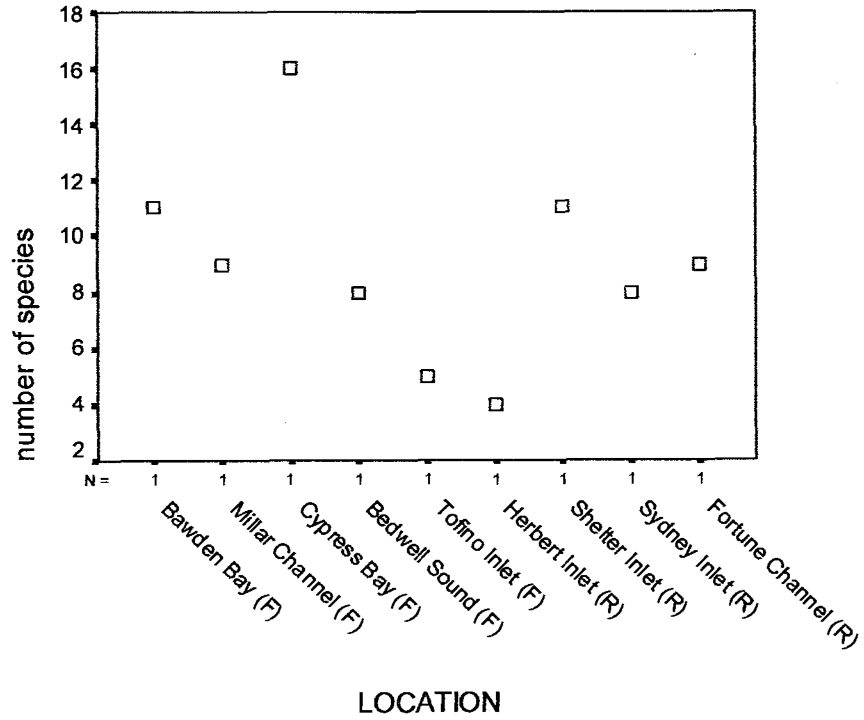


Table 1. Evaluation of the effects of treatment (farm/no farm), location, and distance from farm (sampling station) on average abundance (individuals/m^2) of mid-intertidal invertebrates using nested ANOVA and variance components analysis (sampling station nested within location, location nested within treatment). Each sampling station was the average of three replicate, 0.5m^2 quadrats. Each effect above transect (sampling station, location, treatment) was tested by using the MS of the next lowest factor as the error term.

Variable	Source of variation	df	MS	F	p	Variance component
abundance	treatment	1	6.25×10^8	.281	0.612	0%
	treatment(location)	7	2.31×10^9	9.18	0.000	47%
	location(sampling station)	31	2.54×10^8	1.61	0.05	9%
	error (quadrat)	73	1.58×10^8			44%

Figure 3. Mean (+/-1 SE) total abundance (/m^2) of mid-intertidal invertebrates at five farms (F) and four reference (R) locations in Clayoquot Sound. Mean total abundance for each location was determined from 3-6 sampling stations. Total abundance estimates at each sampling station were determined from three replicate 0.5m^2 quadrats.

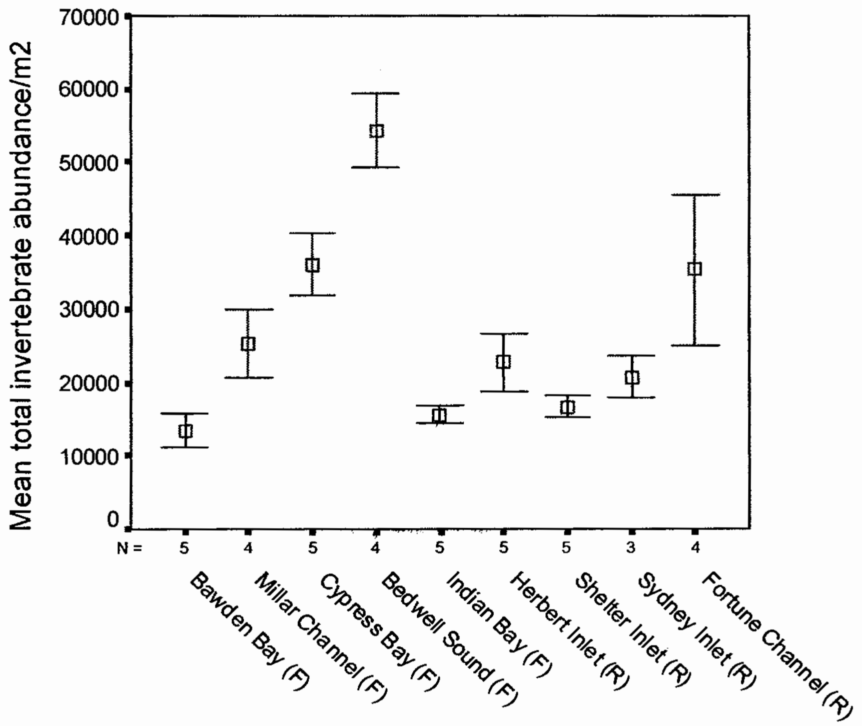
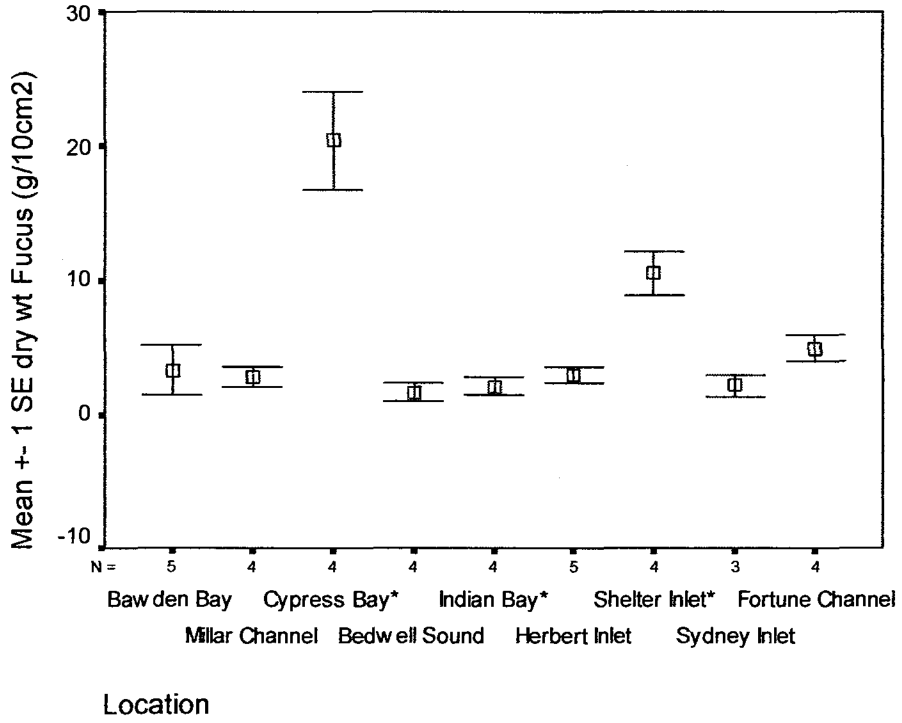


Table 2. Average abundance of mid-intertidal invertebrate predators (individuals/m^2) at five farm (F) and four reference (R) locations in Clayoquot Sound 2002. There is no significant difference in the abundance of predators between farm and reference locations (t= 1.266, df= 7, p= 0.246). Abundance averages were obtained from 3-6 sampling stations per location, sampling station data represents three pooled 0.5m^2 random quadrats.

Location	Average abundance of invertebrate predators/m^2 (*Fucus* dry biomass)	
Bawden Bay (F)	3.4	(3.27)
Millar Channel (F)	1.32	(2.78)
Cypress Bay (F)	5.72	(20.42)
Bedwell Sound (F)	0	(1.67)
Tofino Inlet (F)	0	(2.10)
Herbert Inlet (R)	0	(2.98)
Shelter Inlet (R)	1.32	(10.48)
Sydney Inlet (R)	0.56	(2.16)
Fortune Channel (R)	0	(4.91)

Figure 4. Average dry weight of *Fucus* (g/10cm^2) for each location. (F) = farms, (R) = references. Each location is represented by 3-5 samples.



Discussion

The presence of a salmon farm had no detectable effect on adjacent intertidal community structure. Species richness, total species abundance (total number of individuals) and predator abundance were not different at farms when compared to reference locations. Natural variation at multiple spatial scales and minimal contribution of salmon farm wastes to the intertidal zone are likely explanations. Community structure was associated with natural variation in *Fucus* standing crop.

Natural variation in total abundance of species, species richness and predator abundance at large (kms) and small (meters) scales, likely obscures any slight changes that might result from farm derived subsidies. Small scale (meters) variation explains almost half of the total variation in total abundance of species. Quadrats within locations ranged from nearly bare to densely populated. Farm effects would likely have to be quite large to surpass this magnitude of natural variation. Small scale variation is likely a combination of physical and biological factors. Scouring from winter storms, competition, predation, priority effects (recruitment timing and rates), time since last disturbance, are all factors that are known to contribute to intertidal community structure at small spatial scales (Connell 1961, Dayton 1971, Lubchenco 1976, Menge and Sutherland 1976, Gaines and Roughgarden 1985, Power 1992). Large scale variation could be a product of degree of exposure to the open coast, where significant upwelling occurs and the energy of the system via wave action is higher, delivering nutrients and oxygen at higher rates to the intertidal zone. At Bawden Bay for instance, the sampling station closest to the open ocean is much higher in *Fucus* biomass and has the highest species richness and predator abundance.

Evidence from this study suggests that variation in species richness and predator abundance are a function of a natural productivity gradient, rather than nutrient subsidies from salmon farms. However, species richness at Cypress Bay was the highest of all locations and may be partly explained by very low mussel abundance. It is known that mussels are competitively dominant for space in the intertidal zone (Stephenson and Stephenson 1949, Paine 1973), their removal increases species richness by up to 25 species of invertebrates and algae (Paine 1973). Likely a combination of low mussel density and higher productivity are responsible for the high species richness at Cypress Bay.

Accurate predator abundance estimates might have suffered from inadequate sampling design. Intertidal predators tend to be more active when the tide is covering the intertidal zone (Paine 1973, Menge and Sutherland 1976), seastars and nucellid whelks were aggregated in cracks at low tide or retreated with falling tides, presumably to avoid desiccation and predation. Since all community surveys were conducted at low tides, predator abundance is likely underestimated. However, the fact that I did encounter some predators provides at least a basis for comparison since sampling was conducted in the same way at all locations. Variation in predator abundance will still be attributed to natural variation along a productivity gradient rather than sampling bias.

A critical question is the availability of salmon farm wastes to the intertidal community. Particulate and dissolved wastes originating from the farm may be intercepted by mussels and algae growing on the farm, as well as local phytoplankton (Taylor *et al.* 1995, Cheshuk *et al.* 2003). Integrating the culture of filter feeders (e.g. oysters or mussels) and algae with salmon has been examined as a candidate solution to reducing the amount of wastes released to the surrounding environment (Ahn *et al.* 1998, Troell and Nordberg 1998, Troell et al. 1999). Dilution and dispersion of wastes by currents (Mackas *et al.* 1985), combined with distance of farms from shore could prevent these wastes from reaching the intertidal zone. Additionally, it has been demonstrated that transport of solid wastes is largely vertical and within 60m of the edges of the cages, depending upon local current speed, direction and depth (Brown *et al.* 1987, Kaspar *et al.* 1988, Sutherland *et al.* 2001, Cheshuk *et al.* 2003). In this study, farms ranged 50-400m from shore, thus close to, or outside reported effected zones. The lack of supporting evidence from stable isotope (^{15}N) analysis in another part of this study suggests that at best, farm derived nutrient subsidies to the intertidal zone are small and natural variation prevents detection of slight changes.

When aspects of community structure were correlated with *Fucus* standing biomass, interesting patterns emerged. Species richness and predator abundance were positively and significantly correlated with *Fucus* biomass when all locations were considered together. These results could be explained by both trophic-dynamic models developed by Fretwell (1977) and Oksanen *et al.* (1991) and the unimodal hump-shaped productivity-species richness relationship described by Rosenzweig (1992) and Leibold (1999). The trophic-dynamic model predicts that along an increasing gradient of productivity, eventually additional trophic levels will form as community biomass accumulates. Although a hump-shaped relationship between *Fucus* standing biomass and species richness was not observed in this study, the positive relationship I observed might be the left tail of the "hump", suggesting that the inland waters of Clayoquot Sound are at low to moderate levels of productivity. *Fucus* was the only macroalgal species in many intertidal areas of Clayoquot Sound, indicating that for at least some areas, productivity is low, as *Fucus* is adapted to grow in low nutrient levels where fast growing green algaes cannot (Pedersen and Borum 1996).

Fucus has important direct effects on community structure as a protective canopy, thus providing more habitat and food availability by reducing desiccation for intertidal invertebrates and other edible algae (Menge 1978, Wootton 1991). Protection from desiccation could allow predators to forage more actively (Menge 1978, Wootton 1991). Provision of additional habitat reduces competition among species for space (Abrams 1995), and elevation of resource productivity supports new consumer populations or trophic levels (Fretwell 1977, Oksanen *et al.* 1981, Abrams 1995).

Total species abundance was not correlated with *Fucus* standing biomass. However this may not be an informative result in terms of trophic dynamics. Since productivity can be expressed as abundance or biomass, it is not clear if there was an overall increase in community productivity in terms of growth of existing individuals. In another part of this study, mussel biomass was not found to be affected by proximity to salmon farms.

The intertidal community of Clayoquot Sound covers a large spatial extent, is exposed to nutrient subsidies from salmon farms and encompasses a natural productivity gradient. It appears that this system might be at a low to moderate end of a productivity gradient, supported by the presence of sparsely or unoccupied primary substrate in many areas. There is no evidence supporting a nutrient subsidy from salmon farms to the intertidal community despite large amounts of annual waste production. This is likely due to farms being anchored too far from the intertidal zone, currents diluting wastes, interception of wastes by mussels and algae growing on the farm, and natural variation. However, strong correlations exist between variation in *Fucus* production and species richness and predator abundance. These results support current productivity-species richness hypotheses and trophic-dynamic models.

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Chapter 5

General Discussion

For several decades, studies of marine intertidal communities have provided valuable information about the factors that form the patterns, and the variation, in community structure we observe in nature. Despite logistic difficulties in executing experiments in an open and dynamic system such as the ocean, several studies have employed manipulative experiments (Paine 1973, Dayton 1971, Lubchenco 1978, Duggins *et al.* 1989, Wootton *et al.* 1996), and natural comparative studies along environmental gradients (Bustamante *et al.* 1995 *a,b,* Menge and Sutherland 1976, Menge *et al.* 1997*a,* Nielsen 2001, Menge *et al.* 2003), over a range of spatial and temporal scales. Physical and biological factors have been shown to interact and are variable in strength across spatial and temporal scales. The intertidal community is a good model community for such investigations, as it spans huge spatial scales while remaining fairly conserved in species composition across wide areas (Stephenson and Stephenson 1949, Menge *et al.* 2003), while crossing numerous environmental gradients known to have significant effects on community structure, alone or in combination.

Net-cage salmon farming offers a unique opportunity to investigate the effects of a potential nutrient subsidy to adjacent intertidal communities. Salmon farm wastes are utilized by marine producers and consumers (Ahn *et al.* 1998, Mazzola and Sará 2001, Vizzini and Mazzola. 2004). In British Columbia (BC), farms are generally clustered, thus representing replicable units in a spatially manageable area. The magnitude of nutrients (as excess feed and metabolic wastes) released is quantifiable and can be detected over large spatial scales (100's of meters, Jones and Iwama 1991, Mazzola and Sarà 2001, Vizzini and Mazzola 2004). The residency of a salmon farm in a single location over years also allows for the investigation of changes in population dynamics, species compositions and lagged responses over time.

I investigated the potential for salmon farms to provide a resource subsidy to adjacent intertidal communities, and what effects to community structure might result. I used a comparative approach and a spatially nested sampling design at five farms and four reference locations throughout Clayoquot Sound, an area of concentrated salmon farming on the mid west coast of Vancouver Island, British Columbia. I focused on biomass and stable isotope (^{15}N) content of primary producers and consumers, and community structure of intertidal macroinvertebrates. My predictions were threefold:

1) The presence of a salmon farm would result in increased biomass of intertidal phytoplankton, *Fucus* and mussels, which would be detectable as a gradient; the magnitude of response inversely proportional to distance from the farm.
2) Organisms assimilating salmon farm derived nutrients would reflect this subsidy as being enriched for ^{15}N relative to those without access. This response also inversely proportional to distance from the farm.
3) Invertebrate community structure would reflect this nutrient subsidy as increased abundance of individuals and a shift among species abundances (some species increasing, some decreasing) measured as significantly dissimilar from areas without access to this subsidy.

I found only weak evidence supporting prediction One. Predicted but nonsignificant trends for *Fucus* biomass were detected at two of five farms (Cypress Bay and Indian Bay) and one reference location (Shelter Inlet) that was a recently (7-8 months) fallowed farm. *Fucus* is a perennial algae that can live 5-6 years, thus the pattern of biomass distribution at the fallow farm could be a reflection of previous long-term exposure. Three farms (Cypress Bay, Bawden Bay and Bedwell Sound) showed the average weight of mussels closest to these farms to be larger than those further away, however this result was not significant and the diminishing gradient was not observed. Phytoplankton biomass was highly variable spatially and temporally, but apparently not affected by salmon farm wastes.

No support was found for prediction Two. *Fucus,* eelgrass and mussels growing near salmon farms were not enriched for ^{15}N in comparison to reference locations. $\delta^{15}N$ of mussels collected directly from salmon cages were not different from those collected from pristine areas. *Fucus,* eelgrass and mussels were not different from one another, occupying the same range of 6-10 ‰.

No support was found for prediction Three. Total abundance of organisms was not different at farms in comparison to reference locations, and community composition was not found to change with distance from farms. Species richness and predator abundance were positively correlated with *Fucus* standing biomass in accordance with current species richness-productivity theories, and trophic-dynamic models.

For all variables investigated, there are several common reasons why the predictions of this study were largely unsupported. Minimal nutrient subsidies to the intertidal zone from salmon farms, natural variation at multiple spatial scales, and potential lack of nutrient limitation for focal organisms are the most likely explanations. Limitations of some analyses may also prevent detection of small changes.

Availability of Salmon Farm Derived Nutrients to Intertidal Organisms

A critical question is the availability of salmon farm wastes to the intertidal community in Clayoquot Sound. There is evidence from other studies that effects from farm derived nutrients can be measured up to several hundred meters away. Effects include elevated growth and production of filter feeders (Wallace 1980, Jones and Iwama 1991), increased phytoplankton biomass and POM concentrations (Jones and Iwama 1991, Mazzola and Sarà 2001), and elevated stable isotope signatures (Mazzola and Sarà 2001, Vizzini and Mazzola 2004). However none of these results were observed in this study, suggesting that salmon farm wastes are unavailable, or only minimally available to intertidal communities. Currents can alter the density and distribution of dissolved and particulate nutrients (Mackas *et al.* 1985). This possibility combined with the distance the farm is from shore could interact to dilute salmon farm wastes making their contribution to intertidal resources minimal. Additional dilution of salmon farm wastes is facilitated by virtue of pulsed feeding (and release of metabolic wastes), while intertidal primary producers and filter feeders are utilizing nutrients continuously. Wastes released from salmon farms could be intercepted by other species not considered in this study. Mussels and algae growing on the farm, as well as pelagic phytoplankton likely intercept some farm wastes (Taylor *et al.* 1995, Ahn *et al.* 1998, Troell *et al.* 1999a,b). Additionally, *Fucus* is a poor competitor for dissolved nutrients relative to other species (Wallentius 1984, Worm and Sommer 2000). It is possible that if dissolved salmon farm wastes are available to intertidal algae, that other species are depleting this resource before *Fucus*. This possibility was not considered during data collection.

Natural Variation

Natural variation at multiple spatial scales for all variables likely prevented detection of any small changes contributed by salmon farm wastes. Distribution and abundance of phytoplankton, dissolved and particulate resources depends on a multitude of physical conditions, including current speed and direction, depth, wind speed, and rates of ecological processes such as nutrient cycling, and resuspension (Mackas *et al.* 1980,1985, Menge *et al.* 2003). Physical and oceanographic characteristics of each location (i.e. depth and width of channel/inlet, bathymetry, shoreline morphology) interact with currents and wind to affect nutrient concentration, residence time of water, temperature and salinity (Mackas *et al.* 1980, Thompson 1981, Mackas *et al.* 1985, Duarte 1990, Eslinger *et al.* 2001), all important determinants of phytoplankton and *Fucus* production, and availability of dissolved and particulate nutrients to intertidal plants and suspension feeders (Mackas *et al.* 1985, Daly and Smith 1993, Kocum *et al.* 2002). Variation in these factors will lead to variation in productivity, biomass, abundance, and community structure.

Variability in an organism's isotopic signature is facilitated by differences in isotopic composition of resources and the relative contribution to diet, either by preference or availability (DeNiro and Epstein 1980, Wada 1980, Owens 1987, Peterson and Fry 1987). The relative ^{15}N enrichment and concentrations of dissolved NO_3 and NH_4 pools available to primary producers are variable based on oxygen content of the water (Owens 1987), supply of upwelled water (Mackas *et al.* 1980, Yin *et al.* 1996), input of fresh water (riverine or rain) (Mackas *et al.* 1980, Valiela *et al.* 1992, Page *et al.* 1995) and season (Mackas *et al.* 1980, Daly and Smith 1993, Yin *et al.* 1996). These factors likely contribute to variation in $\delta^{15}N$ of primary producers throughout Clayoquot Sound, as it has rivers of varying flow capacity, is situated next to an upwelling zone to which the various inlets and channels are variably exposed. Additionally, it is unclear whether primary producers are nutrient limited. Resource limitation affects degree of isotopic fractionation. Fractionation is reduced when nitrogen availability is limited and reaction rate exceeds supply rate of substrate (Wada and Hattori 1978, Owens 1987). The nitrogen source is converted entirely to product, and there is no net preference for the heavy or light isotope (Wada and Hattori 1978).

The diet of mussels is variable and is limited by particle size rather than source (Seed 1969, Widdows *et al.* 1979). Dietary items include phytoplankton, zooplankton, organic detritus and solid wastes of intertidal organisms (Seed 1969, Widdows *et al.* 1979, Mazzola and Sarà 2001). There can be considerable variation in $\delta^{15}N$ within and among each of these resources (DeNiro and Epstein 1980, Owens 1987, Duggins 1989), along with the relative abundance of each in the diet of a mussel thus contributing to natural variation in mussel $\delta^{15}N$.

There are many factors that contribute to natural variation in community structure on small and large spatial scales. Predation, competition and disturbances are known to affect intertidal community structure on small spatial scales (Connell 1961, Dayton 1971, Paine 1973, Lubchenco 1978). Nutrients and larval recruitment (Gaines and

Roughgarden 1985, Bustamante *et al.* 1995a, Menge 2003) have influence over larger spatial scales. Phytoplankton and *Fucus* biomass were shown to be spatially variable, species richness and predator abundance were positively and significantly correlated with *Fucus* standing biomass. The inlets and channels of Clayoquot Sound are variably exposed to the open ocean where significant seasonal upwelling occurs and the energy of the system via wave action is higher, thus potentially adding to variation in delivery rates of nutrients, oxygen and larvae from the outer coast.

Are the Focal Species Nutrient Limited?

It is unclear whether primary producers and filter feeders are nutrient limited in Clayoquot Sound. There is evidence that mussels are not food limited, leaving other growth factors to explain the differences among locations for mussel size. Maximum filtration concentrations for mussels 1.7 cm long are 2.6 ug/L seston (Widdows *et al.* 1979, Stirling and Okumus 1995). Evidence from commercial mussel culture operations show that maintenance rations for mussels are 2.4ug/L Chl *a*, but that considerable growth can be maintained at 1.0 ug/L (Stirling and Okumus 1995). The average length of mussels in the present study was less than 1.7 cm long (data not shown). About half the samples in this study met or exceeded 2.6 ug/L of Chl *a* in 2001, and no location was consistently below 1.0 ug/L. Although seston concentration was not measured in this study, Chl*a* concentrations alone appeared to be at feeding saturation levels for at least half the samples in 2001. It is unknown whether non-algal seston elevates total food availability to filtration limits in the remaining samples.

In BC, salmon farms release an average of 740kg waste/day (Naylor *et al.*. 1998, BC Salmon Farmers Association). The instantaneous concentration of waste surrounding the farms (volume roughly calculated as $\pi r^2 h$, where radius= distance to nearest sampling station, h= depth below farm, and assuming even distribution of waste throughout this volume) ranged between 19 and 4355 μg/L. Accounting for settling of solids (Sutherland *et al.* 2001, Cheshuk *et al.* 2003) and distance, if I assume only 0.1% of total wastes released are transported to the intertidal zone, this translates to between 0.019 and 4.3 μg/l of additional resources. Average phytoplankton biomass at each location ranged 0.5-12 μg/L (data from Chapter 2 of this study). Theoretically, the proportional addition of wastes ranges 1-453%, but mostly below 20%. The largest theoretical contribution would be at Tofino Inlet, as it was the closest to shore (52m) and phytoplankton biomass was consistently below 2 μg/L. Tofino Inlet would be the most likely farm to supply the intertidal zone with a resource subsidy, however, no farm effects were detected for any variables. Likely explanations are currents flushing wastes away, and below average annual production.

However, there is also evidence that intertidal organisms may be nutrient limited in some areas. Primary space is known to be the principal limiting resource in intertidal communities but there were many areas of Clayoquot Sound where primary substrate was nearly bare, or common species were absent, implying that nutrients might be important limiting factors. In Millar Channel and Bedwell Sound, *Fucus* was visibly sparse, and no other algal species were present. This observation could indicate that dissolved nutrients are limited, because *Fucus* is adapted to live in low nutrient levels where fast growing filamentous green algae cannot (Pedersen and Borum 1996).

Limitations of the Analyses

Limitations of the analyses chosen for this study could also contribute to an inability to detect a salmon farm effect.

The power analysis gave a clear indication that given the natural variation among locations, the sample sizes were far too small for the observed effect sizes to be significant. The required samples sizes are larger than the number of available farms, thus I conclude with the available samples that any effects are likely to be smaller than the upper 95% confidence limit.

The Bray-Curtis dissimilarity index is not ideal for detecting small changes in species richness or abundance (Krebs 1989). Since only 10-15% of biomass is transferred from one trophic level to the next (Lindeman 1942, Pimm and Lawton 1977), large elevations in productivity would be required to translate into detectable changes in species richness and abundance. There is only weak evidence from this study that salmon farms increase intertidal productivity, expressed as increased biomass of *Fucus* and mussels. Biomass can be increased through growth of individuals or addition of new individuals through reproduction or immigration. Neither the Bray-Curtis analysis nor the ANOVA of total species abundance detected any differences among farms and reference locations.

There is no evidence from this study to suggest that open net-pen salmon farms provide a resource subsidy to adjacent intertidal communities in Clayoquot sound. Biomass of primary producers and mussels, their stable isotope content and invertebrate community structure at farms was not different from reference locations. The stable isotope ^{15}N was not an informative tracer of farm-derived nitrogen. The lack of support for the predictions of this study are likely because farms are anchored too far from the shore, dilution of wastes by currents and pulsed feeding, and natural variation on multiple spatial scales.

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