Proceedings from the University of Washington
School of Oceanography Senior Thesis, Academic Year 2012-2013

Impacts from aquaculture facilities on intertidal ecosystems using quadrant surveys and antibiotic tracers

Ryan D'Jay

[1]*University of Washington, School of Oceanography,*
Box 355351, Seattle, Washington 98195
ryandjay@uw.edu
Received June 2013

NONTECHNICAL SUMMARY

Marine ecosystems are vulnerable to anthropogenic influences due to increased stresses from climate change and habitat destruction. Fish farming and other aquaculture practices have potential impacts on natural marine ecosystems. I examined the effects of fish farms and oyster farms on the health of rocky intertidal communities by quantifying shifts in species richness, biodiversity, and percent coverage near the aquaculture sites in comparison to more natural settings. This research took place in Barkley Sound Vancouver, Canada between January 26th and February 2nd 2013. I hypothesized that impacts of aquaculture, shown by the presence of antibiotics used by them, would decrease biodiversity and species richness in rocky intertidal communities. Observed were impacts from aquaculture sites that caused reduction of biodiversity. The reduction in biodiversity was caused by three different influences. The most intense influence was the introduction of Pacific Oysters to intertidal areas near oyster farms that out competed natural organisms for space. Changes in dissolved oxygen and dissolved organic carbon in the surface waters and the presence of antibiotics were the other two influences observed that altered natural intertidal communities. The impacts were found to possibly extend further distance than expected as the tracers of antibiotics were found at other sites away from the fish farm.

ABSTRACT

This study identifies the impacts from aquaculture facilities on adjacent intertidal ecosystems in Barkley Sound Vancouver, Canada. The influences on intertidal ecosystems examined using intertidal quadrant surveys were biodiversity, species richness, and percent coverage. The influence on water properties that were examined where changes in dissolved organic carbon (DOC), dissolved oxygen, and presence of antibiotics. Antibiotics were also used as a tracer for extent of impact from the aquaculture site. Sites thought to be pristine were used as controls for comparison and intertidal areas next to developments and logging operations were used to determine the severity of the impact. Pacific Oysters, *Crassostrea gigas*, an invasive species to Barkley Sound were only found at oyster farming aquaculture sites. Average total percent coverage fell from 49.1% on control site intertidal areas to 27.5% and 37.75% for fish farming and oyster farming sites respectively. Average invertebrate coverage was highest on intertidal areas impacted by oyster farming at a level of 11.9%. The control sites average invertebrate coverage was 7.56%. Biodiversity calculated using the Simpsons Biodiversity Index showed the overall impact of aquaculture as it dropped from 0.5 in control sites to 0.28 at the fish farm site. Oyster farming sites had the lowest average dissolved oxygen at 58.8%. Base sites had the highest average dissolved oxygen at 67.4%. Antibiotic tracers were found at all aquaculture sites and concentrations fell below 21 ng/L. The extent of antibiotic impacts from aquaculture sites was greater than anticipated.

Growing human populations coupled with the landings of global comercial fisheries leveling off or declining due to fish stocks becoming depleted have created a need for new sources of protein and food (Neori, A. M. et al. 2010). Aquaculture has become the most economic and efficient source in supplying the worlds growing demands and supplies a third of total fisheries production (Primavera, J. H. 2006). Meanwhile natural marine ecosystems are becoming vulnerable to anthropogenic influences due to increased stresses from climate change and habitat destruction (Allison G. et al. 1998). Impacts from aquaculture on marine ecosystems include introduction and transfer of species to new environments, spread of parasites and diseases between farmed and wild stocks, misuse of chemicals, and release of wastes (Primavera, J. H. 2006). Fish farms alone use an array of pesticides and antibiotics that leak out into the natural environment (WU, R. 1995). Antibiotics and pesticides have been shown to influence marine ecosystems near their sources causing changes to biodiversity and algal cover (Boyra, A., and F. J. A. P. Nascimento. 2004). They also show significant effects on the reproduction productivity and health of many marine species (Buschmann, A. H., A. et al. 2012; Carballeira et al 2012; Bris, H. L., and H. Pouliquen. 1995). It is critical to identify the effect of aquaculture activates on marine ecosystems to improve policies and regulations.

Environmental impacts of aquaculture facilities depend greatly on species, culture method, stocking density, feed type, and hydrography (WU, R. 1995). Types of pollutants produced by these farms include organic nutrients, feed waste, artificial feed, vitamins, antibiotics, and escaped stock (WU, R. 1995). Oxytetracycline is one of the most commonly used antibiotics in fish farming (27-74%) and is introduced into the environment via fecal loss, non-ingested food pellets, and renal excretion (Grigorakis, K., and G. Rigos. 2011). Aquaculture sites are often located close to or on coastlines in protected bays and coves. Previous studies have shown that pollutants from fish farms are present 30 to 50 m from their source (Buschmann, A. H., A. et al 2012). Fish farms and the pollutants produced by them have a direct impact on the marine environments surrounding them.

Antibiotics used in fish farms, including Oxytetracycline (OTC) and Tetracycline, have effected marine environments leading to increased resistance of bacterial pathogens of fish (Aoki, 1989; Dixon, 1991). Antibiotics have a direct influence over the types of marine bacteria because they build resistance to antibiotics. Direct influences on marine invertebrates from antibiotics have been studied and observed to include accumulation of antibiotics in filter feeders and influence the growing rates of Sea Urchin Embryo. (Buschmann, A. H., A. et al. 2012; Carballeira et al 2012; Bris, H. L., and H. Pouliquen. 1995).

Aquaculture facilities are often located on or close to shorelines likely influencing intertidal zones. Rocky intertidal areas are sections of coastline that experience exposure during low tides and submersion during high tides (Southward A.J. 1958). They are inhabited by ectotherm invertebrates and algae that are of marine origin but spend large parts of their life in terrestrial environments during low tides **(**Helmuth et al. 2006**)**. With the steep gradient in thermal and desiccation stresses associated with the tidal changes, the rocky intertidal zone has served as a natural lab to study relationships between abiotic stresses (Bertness et al. 1999; Connell 1972; Paine 1994; Somero 2002; Southward 1958; Wethey 1984).

It has been shown that fish farms effect biomass and percent cover of intertidal communities (Boyra, A., and F. J. A. P. Nascimento. 2004). The pollutants of fish farms have been seen to increase the presence of algae cover, in both red and green algae, over the intertidal area. Broya and colleagues suggest that this could be caused by the increase in organic matter released from the fish farms (Boyra, A., and F. J. A. P. Nascimento. 2004). I hypothesized that antibiotics, in addition to organic matter, lead to decreased invertebrate diversity and increased algae cover of the intertidal communities. Studies of the effects of shellfish farming on benthic communities show little impact in relation to fish farming (Crawford, C. M. et al. 2003). Oyster farms however have shown to increase macro fauna biomass, abundance and number of but not on a constant basis (Mallet, A. L et al. 2006). Mallet et al concluded that environmental impacts of oyster culture should be assessed on other parameters then sediment redox and sulfide levels and that there is possibly more impact shown in the surrounding ecosystems.

The purpose of this research was to look into the impacts of aquaculture sites on adjacent intertidal ecosystems. Traces of antibiotics were used as a direct link connecting fish farming and oyster farming to intertidal systems and the possible impact of the pollutants. Intertidal surveys were conducted to examine changes in spices richness, biodiversity, percent coverage and the shifts that may occur from pristine sights to ones near aquaculture facilities.

METHODS

Field Work

All samples were collected in Barkley Sound on Vancouver Island, Canada (48 50'N, 125 10'W) from small boats deployed from the *R/V Thomas G Thompson* between 27th of January and the 1st of February in 2013. Thirteen intertidal sites were surveyed (Fig. 1). Three "base" sites thought to represent intertidal areas in pristine conditions and were used as controls. Three logging sites were sampled represented intertidal areas impacted by nearby logging operations. Two development sites were sampled represented intertidal areas impacted by nearby cabins and houses. One non active fish farm was found and sampled representing an intertidal area influenced by fish farming. Four aquaculture sites were sampled representing intertidal areas affected by aquaculture in the area all being line Oyster farms. Samples were collected as close to low tide as possible. A 1m x 1m quadrant divided up into 100 $10cm^2$ sections was used to conduct intertidal surveys of biological communities. Nine quadrants were sampled randomly taking 3 in the upper intertidal zone, 3 in the middle intertidal zone, and 3 in the lower intertidal zone. Pictures were taken of each quadrant sample using a Cannon Powershot camera to be examined at a later date.

Water samples were collected near the shore from a small boat. Water samples were to use to observe concentrations of antibiotics and dissolved organic carbon (DOC). Water was collected using a hand held Niskin bottle at 5m depth. Two liters were collected for antibiotic samples and volume of water collected for DOC was in accordance to the practices of the Krogsland lab at University of Washington. Triplicates were taken at three stations (Aq_10, AQ_11,and AQ_12) (figure 1). Temperature, salinity and dissolved oxygen were gathered using a YSI probe. Presence of *Pisaster ochraceus* was observed from the small boat.

DOC samples were frozen in -60 freezers until they could be sent to the Krogsland lab for processing. Antibiotic water samples were kept in dark fridge until they could be processed within 24 hours on board the ship. Samples were filtered through– a 0.45 µm filter, to remove suspended particles that would inhibit Solid Phase Extraction (SPE) (Vazquez-Rogi, P. et al .2011). Samples were then processed for later SPE in the methods described by Vazquez-Rogi, P. et al (2011).

Figure 1: Map of sampling locations of intertidal sites in Barkley Sound located on Vancouver Island, B.C. Canada.. Base sites shown by green dots represent pristine conditions. Log sites shown by black triangle represent intertidal areas near logging farms. Dev sites shown by black hexagon represent intertidal areas next to cabins and housing. FF site shown by blue asterisk represents intertidal areas near fish. Aq sites shown by black asterisk represents intertidal areas near oyster farming site

Lab Work

Intertidal photos were processed after the cruise. At each station three photos were processed to identify organisms present. Randomly selected were one high intertidal, one middle intertidal, and one lower intertidal photos. Each $10cm^2$ section was classified by what organism or feature was most dominant. Each section represents 1% of the total coverage of the quadrant. Organisms were identified as well as possible using intertidal handbooks and websites. Biodiversity was determined using the Simpsons Biodiversity Index using the percent coverage of each organism identified.

Antibiotics were processed in the Keil lab at the University of Washington (Keil R., and J Neibauer. 2009). Samples were eluted using 6ml ethylacetate and 3ml methanol. Samples were then passed through the cartridges at a rate of 0.05ml/sec. Samples were then dried down under a turbovac producing a gentle stream of nitrogen. Samples were then concentrated down to 1ml samples in a 75:25 water to methanol solvent. Standards were made for Ciprofloxacin, Norfloxacin, Oxytetracycline, Sulfamethoxazole, and Tetracycline. Concentrations were 7.5, 450, 1875, 3750, 5625, and 7500 ng/ml. Samples and standards were then run on a ABI SCI-EX 3200 LC-MS/MS in accordance to the study of Vazquez-Rogi, P. et al in the paper Assessment of the occurrence and distribution of pharmaceuticals in a Mediterranean wetland (L'Albufera, Valencia, Spain) by LC-MS/MS (Vazquez-Rogi, P. et al. 2011).

RESULTS

Intertidal Ecosystem

The most dominant organism found in Barkley Sound was *Fucus vesiculosus* known as Rockweed. Other flora found were *Lithothamnion sp.* known as Pink Encrusting Coralline Algae, *Mastocarpus papillatus* known as Turkish Washcloth and unidentified green algae, white lichens, beach grasses and brown kelp. Invertebrates found in Barkley Sound were *Balanus Glandula* known as Acorn Barnacles, *Mytilus edulis* know as Pacific Blue Mussels, and *Crassostrea gigas* known as Pacific Oysters. The key stone predatory sea star *Pisaster ochraceus* was found at all stations but only below the water line during the time of day the study was able to take place so abundance was unable to be determined.

Total coverage represents the percent area of the intertidal zone covered by both flora and fauna. Intertidal sites affected my developments showed the highest total coverage with an average of 71.3 % (Fig. 2B). The lowest total percent coverage belonged to sites affected by aquaculture. Intertidal area affected by fish farming had a 27.5% coverage and intertidal areas affected by oyster faming had an average of 37.75% coverage (Fig. 2B). Both types were below the total percent coverage on pristine intertidal areas were an average of 49.1% was found (Fig. 2B).

Intertidal sites affected my oyster farms had the highest average percent coverage of invertebrates with 11.9% (Fig. 2C). Percent invertebrate coverage on pristine intertidal areas were an average of 7.56% (figure 2C). The intertidal area affected by the fish farm has the lowest invertebrate coverage with 0.5% (figure 3). Only intertidal areas affected by oyster farms had Pacific Oysters present in their intertidal area with a percent coverage of 8.3% (figure 2C).

Species richness ranged from an average of 3.7 in intertidal areas affected by logging to 5 in intertidal areas affected by development (figure 2A). Standard deviation for species richness across all site types was 1.45. Biodiversity on the Simpsons Diversity Index ranged from an average of 0.50 in the control sites to 0.28 at the fish farm site (figure 2A). The average of 0.39 for sites adjacent to oyster farms was the second lowest (figure 2A).

Water Properties

Water temperature and salinity were stable across the averages of the 5 different station types (Fig. 3A). Dissolved organic carbon (DOC) dissolved oxygen varied greatly between station types. Stations near oyster farming sites had the lowest dissolved oxygen (58.8%) (Fig 3B). Base sites had the highest average dissolved oxygen (67.4%). Stations near oyster farming had higher DOC (89.0 µmol) then pristine sites (72.9 µmol) (Fig 3B). Sites affected by development had the highest DOC (104.5 µmol). Sites affected by fish farming had the lowest DOC (72.1 µmol) (Fig. 3B)

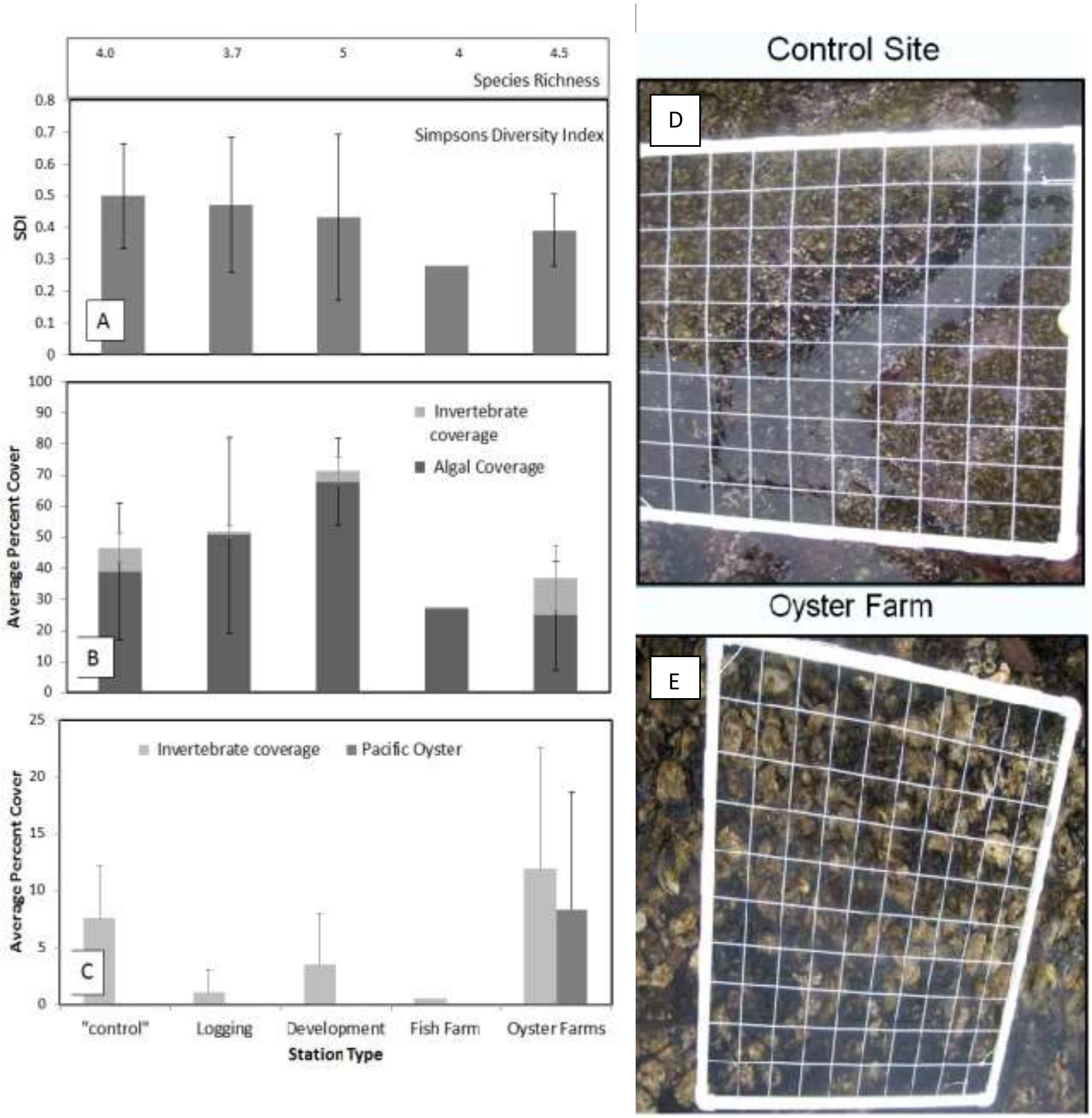

Figure 2: (A) Average Species richness and Simpsons Biodiversity Index of each different type of influence. Species diversity stayed consistent while biodiversity decreased at sites affected by aquaculture when compared to the control sites in more pristine conditions. (B) Averaged percent coverage for each different station type Sites influenced by aquaculture had the lowest average percent total coverage. Sites influenced by oyster farms had the highest average percent invertebrate coverage. (C) Average percent cover of invertebrates and oysters for each type of station. Oyster farming sites had the highest invertebrate coverage and are also the only sites with Pacific Oysters present.(D) Picture of lower intertidal area quadrant at a control site. Present was high rock weed coverage as well as high biodiversity. (E) Picture of lower intertidal area at an oyster farming site. Pacific Oysters are the only organism present.

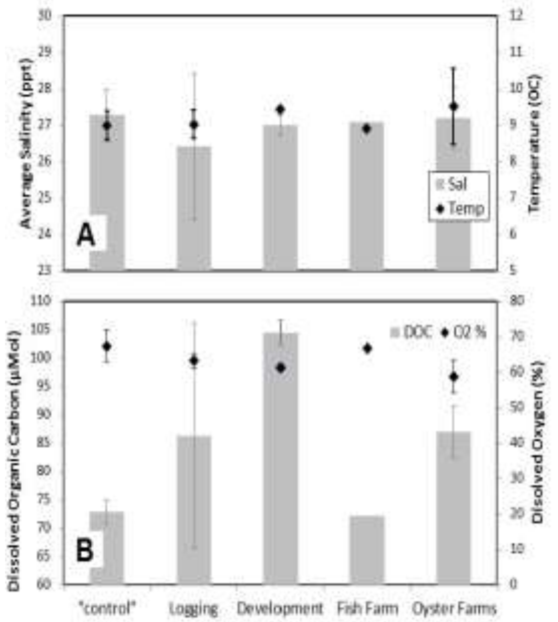

Figure 3: (A). Average salinity in PPT and average temperature in °C for each type of intertidal area. Across the 5 different types of study locations there is no strong changes in both average temperature or salinity.(B) Average dissolved organic carbon (mMol) and average percent dissolved oxygen for each type of intertidal station. Oyster farming sites show lowest percent dissolved oxygen. Development sites have the highest dissolved organic carbon.

Pollutant data

Concentrations of all five antibiotics, Ciprofloxacin, Norfloxacin, Oxytetracycline, Sulfamethoaxole, and Tetracycline fell below 21 ng/L at a specific site (Fig. 4B). Averages of each antibiotic concentration for each site are shown in figure 4B. The highest average concentrations of Ciprofloxacin, Norfloxacin, Oxytetracycline, and Tetracycline were found at the control sites (Fig. 4B). Fish farming had the second highest concentration of antibiotics followed by the average concentration in the oyster farm sites (Fig 4B). Concentrations at each site plotted on a spatial scale using spatial clustering are show in figure x. Sites Base_4, Log_5, Log_6,and Log_9 all had no concentrations of any single antibiotic above 2.8 ng/l (Fig 4C).

Discussion

Shorelines affected by fish farming and oyster farming did not influence the intertidal ecosystems in distinguishable ways. Trends however were observed. Species richness was similar across all types of study sites biodiversity decreased from pristine conditions when influenced by aquaculture (Fig. 2A). Total percent coverage also decreased when impacted by aquaculture facilities was adjacent to the intertidal area (Fig. 2B). The combination of decreased biodiversity and total coverage while species richness stays the same suggest an impact on the intertidal ecosystem (Mallet, A. L et al. 2006). An environmental impact on marine ecosystem is used to state that there has been an alteration or destruction of a natural ecosystem from an outside source. The outside source for these alterations is the aquaculture facilities. The impact could be that an organism is being introduced and out competing natural habitants (Molnar, J. L. et al. 2008). Another possible impact could be the influence on physical properties that would reduce the ability of some organisms to survive (Buschmann, A. H., A. et al. 2012).

The intertidal areas influenced by the oyster farms in Barkley Sound showed the presence of Pacific Oysters (Fig 2E). The Pacific Oysters were not found at any other site surveyed and could be the direct impact of the oyster farms introducing a species not commonly found in Barkley Sound (Fig 2D) (Moehler, J. 2011). The Pacific Oysters seem to be out competing the natural flora and fauna of the area. The introduced competition for space by an introduced species probably caused these areas to have the highest amounts of invertebrate coverage, which was dominated by Pacific Oysters, and lowest percent algal coverage (Caro, A. et al. 2011).

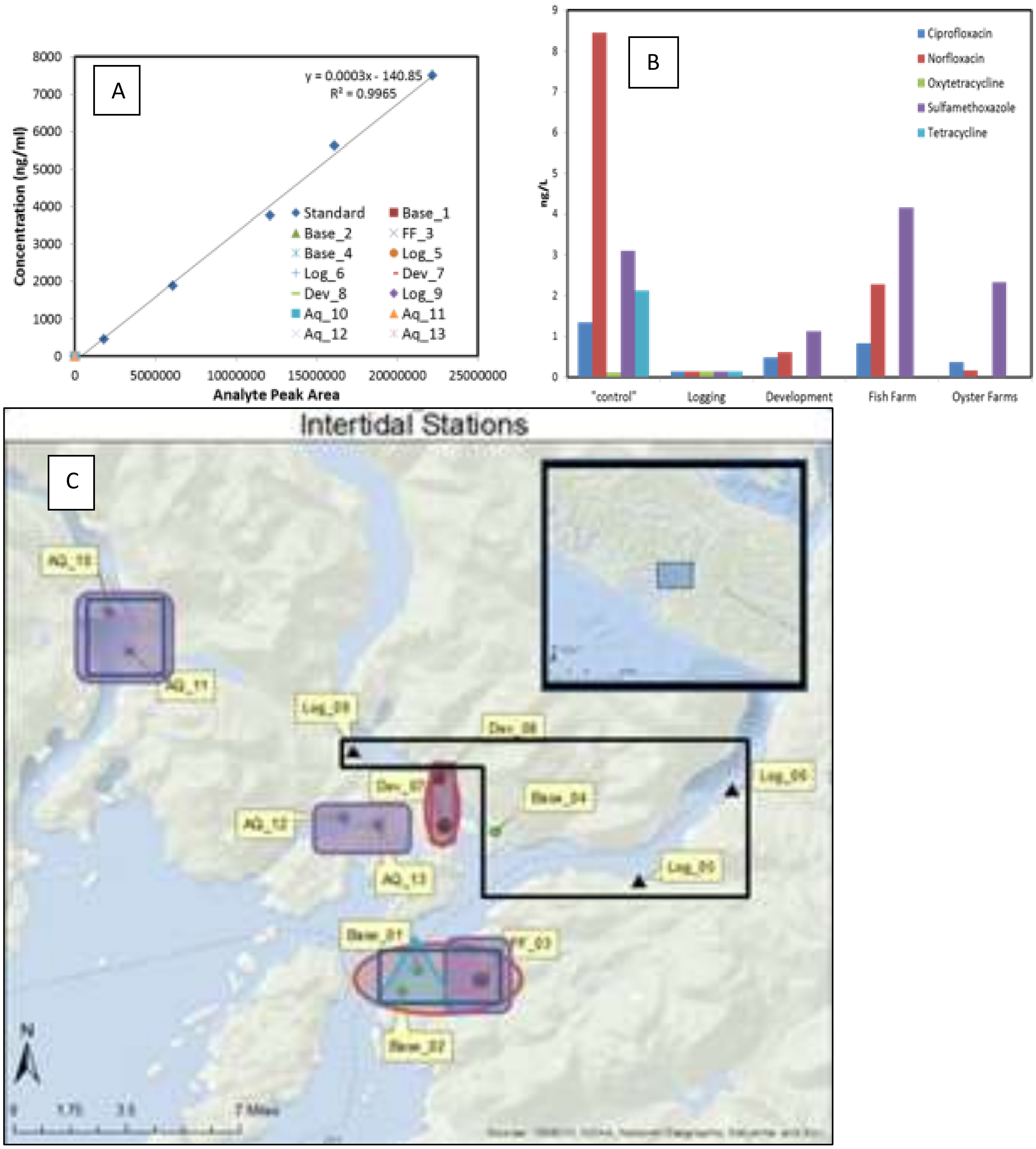

Figure 4: (A) Standard curve and concentrations at study sites of Ciprofloxacin with transition 332.2/231.2 graphed. Analyte peak area against concentration in ng/ml. Concentrations for all the study sites fell below the 7.5 ng/ml standard but were detected due to the sensitivity of the ABI SCI-EX 3200 LC-MS/MS. (B) average concentration in ng/L for each Ciprofloxacin, Norfloxacin, Oxytetracycline, Sulfamethoaxole, and Tetracycline at each different type of study site (C) The spatial distributions of each antibiotic. Dark blue boxes represent the area Ciprofloxacin is present. Red shaded ovules represent the area where Norfloxacin is present. Three were no significant presence of Oxytetracyline. Purple shaded squares represent where Sulfamethoaxole is present. Blue shaded triangle represents where Tetracycline is present. Black box out lines the study locations with no real presence of antibiotics. Base 4 was clean when compared to Base 1 and 2.

The intertidal area adjacent to the non-active fish farming facility had the lowest total coverage with no invertebrate, including Pacific Oyster, coverage (Fig. 2B). With temperature and salinity being similar across the different types of stations the changes in DOC and percent dissolved oxygen must be caused from an outside influence (Fig. 3) (Gilbert, W. E. et al 1968). The low percent oxygen at the oyster farming sites is probably caused by the increased biological activity in the area which may also be the cause of increased organic carbon (Fig 3B) (Boyra, A., and F. J. A. P. Nascimento. 2004; Wu, R. S. S. et al. 1994). The station near the non-active fish farm is similar in DOC and dissolved Oxygen to the pristine base sites (Fig 3B). If fish were added to the pens this would bring down the dissolved oxygen and raise the DOC (Wu, R. S. S. et al 1994). The DOC impacts from oyster farming are not as intense as from development and are about average with logging in the area (Fig. 3B). The decrease in biodiversity and percent coverage at the fish farm site though was greater than the averages of the oyster farms showing that there is some impact. The level of change on the intertidal areas not following the changes in DOC and dissolved oxygen suggest that impacts may further be controlled by pollutants. The pollutants produced by aquaculture practices were used to try and quantify this impact.

Antibiotics were found to be present in Barkley sound in very low concentrations but the ABI SCI-EX 3200 LC-MS/MS was sensitive enough to be able to pick them up (Fig. 4A). Low concentrations of antibiotics are typical of surface waters near aquaculture facilities usually falling below 9 ng/L but have been recorded as high as 50 ng/L (Hirch, R. 1999). The highest average concentration was 8.4 ng/L of Noroloxacin found in the control sites (Fig. 4B). The high level of antibiotics present at the control site led to the need for spatial analysis of the concentrations. It appears the average high levels of antibiotics found for the control site are being driven by the concentrations from Base_1 and Base_2 as Base_4 had the lowest concentrations of all the antibiotics compared to all 13 different sites. This indicates that even though stations Base_1 and Base_2 were initially thought of to be far enough away from the fish farm to be considered pristine that they were in fact influenced by the fish farm (Fig 4C). The antibiotics traveled away from the farm a greater distance then expected to affect these sites because antibiotics are not found to be easily biodegradable and have unknown residency times (Alexy, R. 2004, Hirsch, R et al. 1999). The fish farm had lower concentrations because it had been out of use probably for a few months due to the winter timing and had a chance to clean its system out. This is supported by the fact that the dissolved oxygen and organic carbon levels are similar to those of the pristine sites.

Antibiotics seem able to be used as a tracer for the impacts of Aquaculture in this study. Antibiotics were mainly present in significant levels outside of areas that were with in close range of aquaculture facilities (Fig 4C). There were a few occasions where they were found at the Development sites as well. This is not surprising as these are human used antibiotics and could have been introduced to the system from wastewater runoff from the cabins and houses (Alexy, R. 2004). Sulfamethoaxole could be considered the best tracer of the 5 antibiotics because it is found at every aquaculture site including both the fish farm and oyster farms and nowhere else (Fig. 4C).

These antibiotics, which were being used as tracers, may also be the cause of shifts in reduced algal coverage and total coverage for areas impacted by aquaculture sites. While competition from invasive species and changes in dissolved oxygen and DOC are already causing influences on the intertidal systems, antibiotic presence will increase stress on the algal organisms present (Nickell, L. et al. 1954). It has been discovered that in different concentrations antibiotics can either inhibit or stimulate algal growth (Nickell, L. et al.1954).

The combination of tracers from aquaculture being present and observed shifts in biodiversity, total percent coverage, percent invertebrate coverage, and presence of oysters show that aquaculture is influencing intertidal ecosystems. It is also suggested that these influences may be greater than the impacts of development and logging due to the aquaculture sites having lower levels of biodiversity and coverage. These influences could possibly be extremely harmful to natural ecosystems. The introduced species, Pacific Oyster, is an exotic species to the area and has been know influence intertidal ecosystems in Barkley Sound (Ruesink, J. L. 2007). There is a connection between the changes in water properties and active aquaculture as well as antibiotic presence. There is the possibility that these influences will become more intense as the intensity of the aquaculture activities increase (Boyra, A., and F. J. A. P. Nascimento. 2004). Quantifying the possible impacts from aquaculture on adjacent marine ecosystems like the intertidal zone should be taken into account as demand for aquaculture continues to grow.

Conclusion

Influences from aquaculture sites on intertidal areas were studied and quantified. Trends showing impacts from aquaculture on intertidal ecosystems were observed. Aquaculture sites caused decreased total coverage and algal coverage while also causing reduction in biodiversity of the intertidal ecosystems. Oyster farms also impacted the local intertidal areas by introducing the Pacific Oyster that would out compete the natural flora and fauna. Water properties of dissolved organic carbon and dissolved oxygen were also affected by the presence of aquaculture. The increased biological activity caused lower dissolved oxygen levels and increased dissolved organic carbon. The antibiotics used in aquaculture can be used as a chemical tracer to determine the reach of the impacts from these facilities. Antibiotics may also impact algal growth.

ACKNOWLEDGEMENTS

I would first like to thank my mentors Rick Keil and Bob Morris who read all my drafts many of which were sent in the very early hours. I would then like to thank the rest of the faculty and T.A.s of the oceanography senior thesis class for all their contributions. Thank you to the University of Washington School of Fisheries faculty that helped me in my research as I was very lost on how to do intertidal ecology work. I am an oceanographer as were my mentors and it’s strange to study things bigger then plankton. Thank you to my peers in my department. You have put up with me for five years and even still offer to help. Thank you to my family for their support and love along this whole process. I would like to thank Jaqui Neibauer for all the help in the lab. Thank you to the Sachs Lab for their help as well. I would like to thank caffeine and good music; never would I have made it through the all-nighters without you. Finally I want to thank Pacific Oysters, not only are delicious but you also gave my paper a reason to be written.

REFERENCE LIST

Alexy, R., T. Kümpel, and K. Kümmerer. 2004. Assessment of degradation of 18 antibiotics in the Closed Bottle Test. Chemosphere **57**: 505–12.

Allison G, Lubchenco J, Carr M. 1998. Marine Reserves are Necessary but not Sufficient for Marine Conservation. Ecol. Appl., **8**: S79 –S92

Bertness MD, Leonard GH, Levine JM, Bruno JF. 1999. Climate-driven interactions among rocky intertidal organisms caught between a rock and a hot place. Oecologia **120**:446-50

Boyra, A., and F. J. A. P. Nascimento. 2004. Impact of sea-cage fish farms on intertidal macrobenthic assemblages.Mar. Biol **84**: 665–668.

Bris, H. L., and H. Pouliquen. 1995. Preliminary study on the kinetics of oxytetracycline in shellfish exposed to an effluent of a land-based fish farm: Experimental approach. Mar. Enviorn. Res. **40**: 171–180.

Buschmann, A. H., A. Tomova, A. López, M. a Maldonado, L. a Henríquez, L. Ivanova, F. Moy, H. P. Godfrey, and F. C. Cabello. 2012. Salmon aquaculture and antimicrobial resistance in the marine environment. PloS one **7**: e42724.

Carballeira, C., M. R. De Orte, I. G. Viana, T. a Delvalls, and a Carballeira. 2012. Assessing the toxicity of chemical compounds associated with land-based marine fish farms: the sea urchin embryo bioassay with Paracentrotus lividus and Arbacia lixula. Arch. Environ. Con. Tox. **63**: 249–61.

Caro, A., R. Guiñez, V. Ortiz, and J. Castilla. 2011. Competition between a native mussel and a non-indigenous invader for primary space on ¬intertidal rocky shores in Chile. Mar. Ecol-orog. Ser. **428**: 177–185.

Connell JH. 1972. Community interactions on marine rocky intertidal shores. Annu. Rev. Ecol. Syst. **3**:169-92

Crawford, C. M., C. K. . Macleod, and I. M. Mitchell. 2003. Effects of shellfish farming on the benthic environment. Aquaculture **224**: 117–140.

Dixon, B. A. 1994. Antibiotic Resistance of Bacterial Fish Pathogens. J. World. Aquacult. Soc., **25**: 60–63. doi: 10.1111/j.1749-7345.1994.tb00805.x

Gilbert, W., W. Pawley, and K. Park. 1968. Carpenter's Oxygen Solubility Table and Nomograph for seawater as a Function of Temperature and Salinity,.

Grigorakis, K., and G. Rigos. 2011. Aquaculture effects on environmental and public welfare - the case of Mediterranean mariculture. Chemosphere **85**: 899–919.

Helmuth, B. Mieszkowska, N, Moore, P, and Hawkins, S. 2006. Living on the Edge of Two Changing Worlds: Forecasting the Responses of Rocky Intertidal Ecosystems to Climate Change. Annu. Rev. Ecol. Evol. S. **37.1**: 373-404

Hirsch, R., T. Ternes, K. Haberer, and K. L. Kratz. 1999. Occurrence of antibiotics in the aquatic environment. Sci. Total Environ. **225**: 109–18.

Keil R., and J Neibauer. 2009. Analysis of Cooking Spices in Natural Waters, Limnol Oceanog-Meth. **7**: 848-855.

Mallet, A. L., C. E. Carver, and T. Landry. 2006. Impact of suspended and off-bottom Eastern oyster culture on the benthic environment in eastern Canada. Aquaculture **255**: 362–373.

Moehler, J., K. M. Wegner, K. Reise, and S. Jacobsen. 2011. Invasion genetics of Pacific oyster Crassostrea gigas shaped by aquaculture stocking practices. J. Sea. Res. **66**: 256–262.

Molnar, J. L., R. L. Gamboa, C. Revenga, and M. D. Spalding. 2008. Assessing the global threat of invasive species to marine biodiversity. Front. Ecol. Environ. **6**: 485–492.

Neori, A., M. Troell, T. Chopin, C. Yarish, and A. Critchley. 2010. Environment : Science and Policy for Sustainable Development The Need for a Balanced Ecosystem Approach to Blue Revolution Aquaculture. 37–41.

Nickell, L. G., and A. C. Finlay. 1954. Antibiotics and Their Effects on Plant Growth.J. Agric. Food. Chem. **2**: 178–182.

Primavera, J. H. 2006. Overcoming the impacts of aquaculture on the coastal zone. Ocean Coast Manage **49**: 531–545.

Ruesink, J. 2007. Biotic resistance and facilitation of a non-native oyster on rocky shores. Mar. Ecol-prog. Ser. **331**: 1–9.

Southward AJ. 1958. Note on the temperature tolerances of some intertidal animals in relation to environmental temperatures and geographical distribution. J. Mar Biol. Assoc. U.K. **37**:49-66

Vazquez-Rogi, P. Andreu, V. Onghena, M. Blasco, C. and Pico, Y. 2011. Assessment of the occurrence and distributionof pharmaceuticals in a Mediterranean wetland(L'Albufera, Valencia, Spain) by LC-MS/MS. Anal. Bioanal. Chem. **400**: 1287-1301

WU, R. 1995. The Environmental Impact of Marine Fish Culture: Towards a Sustainable.Future. Mar. Pollut. Bull. **31**: 159-166

Wu, R. S. S., K. S. Lam, D. W. MacKay, T. C. Lau, and V. Yam. 1994. Impact of marine fish farming on water quality and bottom sediment: A case study in the sub-tropical environment. Mar. Environ. Res. **38**: 115–145.